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Performance of Juvenile and Ongrowing  
Common Dentex (*Dentex dentex*, L. 1758, Sparidae)  
in Relation to Nutrition under Culture

von

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## ABSTRACT

Common dentex (*Dentex dentex*) were reared from egg stage to marketable size in a flow-through system at ambient conditions during a three years period (1992 - 1994). A series of rearing experiments were conducted in order to determine biological traits important for the evaluation of this potential aquaculture candidate. In juveniles (2.5 - 50 g), growth rates were found to be exceptionally high compared to other aquaculture species and food conversion very efficient ( $FC \approx 1$ , based on food dry matter). Results were attained under ambient conditions (Temperature:  $\approx 25^\circ\text{C}$ ; Salinity: 40 - 42 ppt). Feeding consisted of moist diets (35%  $\text{H}_2\text{O}$ ) with high energy (22 - 24 MJ/kg) and high protein (51-53 %) content. Feeding exclusively commercial dry pellets (isocaloric to the moist diet), resulted in significantly reduced growth rates, reduced food conversion efficiencies, increased mortalities due to agonistic behaviour and an increased disease susceptibility. The poor performance of test specimens fed dry diets compared to those fed self - prepared moist diets was not explained by the different moisture content of the test diets (8-10% vs 35-50%). Common dentex reach marketable size (300 - 500 g) within 14 - 18 months after hatching under favourable ambient conditions (South Aegean Sea), while feeding self - prepared moist diets. This is in the range of established aquaculture species in the Mediterranean Sea.

The possibility to protect common dentex against losses due to an infectious disease by oral application of different immunostimulants ( $\beta$ -1,3/1,6 glucans; MacroGard™, VitaStim™) was tested in this study. Disease outbreaks are not securely prevented, but treated test groups showed lower overall mortality, while subsequent mortalities were clearly related to treatment.

The study analyze 9 morphometric characters (total length, standard length, fork length, pre- and post anal length, head length, body height, mouth opening, eye diameter) and describes their allometric development in juvenile and adult dentex ( $n = 69 - 463$ ). The occurrence of abnormalities was monitored and described during all rearing periods. Seven types of skeletal deformations and one abnormal scale development were identified. One incident of gas bubble disease, caused by a gas supersaturation of 110 % is described. A swimbladder stress syndrome (SBSS) was assumed to occur during the acclimation of wild broodstock-fish to conditions in captivity.

## Kurzfassung

Während eines dreijährigen Untersuchungszeitraumes (1992 - 1994) wurden Zahnbrassen (*Dentex dentex*) in einem Durchflußsystem vom Ei-Stadium bis zu Tieren von Marktgröße aufgezogen. Zur Charakterisierung von arttypischen Eigenschaften, welche in Bezug auf die Eignung einer Art für die Aquakultur von Bedeutung sind, wurde eine Reihe von Versuchen durchgeführt. Außergewöhnlich hohe Wachstumsraten im Vergleich zu bereits etablierten Aquakulturarten, sowie eine hocheffiziente Nahrungsverwertung ( $FC \approx 1$ , auf Trockenfuttermasse basierend), wurde bei juvenilen Tieren (2.5 - 50 g) festgestellt. Diese Ergebnisse wurden bei Wassertemperaturen von etwa  $25^\circ\text{C}$ , sowie der Verwendung eines Naßfutters (35 %  $\text{H}_2\text{O}$ ) mit hohem Energie (22 - 24 MJ/kg), als auch hohem Proteingehalt (51 - 53 %) erzielt. Im Vergleich zu den mit Naßfutter erzielten Leistungen, führte die ausschließliche Fütterung mit kommerziell verfügbaren Trockenfuttersorten (isokalorisch zu dem Naßfutter) zu signifikant niedrigeren Wachstumsraten, reduzierter Nahrungsverwertung, erhöhten Mortalitäten durch Aggressivität, sowie einer erhöhten Infektionsanfälligkeit. Die relativ schlechte Verwertung von Trockenfutter im Vergleich zu dem selbsthergestellten Naßfutter konnte nicht auf deren stark

Unterschiede im Wassergehalt zurückgeführt werden (8-10% vs 35-50%). Zahnbrassen erreichen etwa 14 - 18 Monate nach dem Schlupf die typische Vermarktungsgröße von 300 - 500 g (unter Temperaturbedingungen im südägäischem Meer). Dies entspricht Abwachsgeschwindigkeiten von bereits etablierten Aquakulturfischen aus dem mediterranen Raum.

Die Möglichkeit, Zahnbrassen durch verschiedene Immunostimulantien ( $\beta$ -1,3/1,6 Glucane; MacroGard™, VitaStim™), die dem Futter beigemischt werden, vor Verlusten durch infektiöse Erkrankungen zu schützen, wurde in dieser Arbeit geprüft. Der Ausbruch von infektiösen Erkrankungen konnte durch die Behandlung mit Immunostimulantien nicht vorgebeugt werden, die Sterblichkeit in den mit Immunostimulantien behandelten Testgruppen war jedoch erheblich reduziert.

Zwischen 9 morphometrischen Merkmalen (Gesamt-, Standard-, Gabel-, Pre-, Post- Anal-, Kopf- länge, Körperhöhe, Maulgröße, Augendurchmesser) wurden Regressionsbeziehungen aufgestellt, als auch allometrische Wachstumsmuster von juvenilen und adulten Zahnbrassen ( $n = 69 - 463$ ) beschrieben. Sieben Typen von Skelettdeformationen und eine Schuppenanomalie wurden charakterisiert. Ein Fall von Gasblasentrauma bei zweijährigen Fischen, verursacht durch eine Gesamtgassättigung von 110 % wurde dokumentiert. Die Anpassung von Wildfängen (Elterntiere) an die Haltungsbedingungen in Gefangenschaft führte zu Verhaltensmustern, die auf ein Schwimmblasen-Streßsyndrom hinwiesen.

## ΠΕΡΙΛΗΨΗ

Σε μια σειρά πειραμάτων συνολικής διάρκειας τριών ετών, πραγματοποιήθηκε η εκτροφή της συναγρίδας (*Dentex dentex*) από το στάδιο των αυγών μέχρι το εμπορεύσιμο μέγεθος. Ο σκοπός της μελέτης ήταν ο προσδιορισμός των απαραίτητων βιολογικών παραμέτρων της συναγρίδας για την αξιολόγηση της ως νέο είδος στον τομέα της Μεσογειακής ιχθυοκαλλιέργειας.

Στο στάδιο των ιχθυδίων (2.5-50 g βάρος) η συναγρίδα παρουσίασε εξαιρετικά υψηλό ρυθμό ανάπτυξης σε σχέση με άλλα είδη, και πολύ ικανοποιητικό δείκτη μετατρεψιμότητας (Δ.Μ.Μ1, υπολογισμένος ως προς το ξηρό βάρος της τροφής). Η διατροφή των ψαριών βασίστηκε σε νωπή τροφή (moist pellet, 35% περιεκτικότητα σε υγρασία) υψηλού ενεργειακού (22-24 MJ/Kg) και πρωτεϊνικού (51-53%) περιεχομένου. Η αποκλειστική διατροφή των ιχθυδίων με βιομηχανική ξηρή τροφή (θερμιδικά ισοδύναμη με τη νωπή τροφή) οδήγησε σε στατιστικά σημαντική μείωση του ρυθμού ανάπτυξης, αύξηση του δείκτη μετατρεψιμότητας, αυξημένη θνησιμότητα λόγω επιθετικής συμπεριφοράς, και αυξημένη ευαισθησία στις ασθένειες. Οι παραπάνω διαφορές δεν μπορούσαν να ερμηνευτούν-εξηγηθούν από τη διαφορετική περιεκτικότητα σε υγρασία. Υπό ευνοϊκές φυσικές περιβαλλοντικές συνθήκες (περίπου 25°C θερμοκρασία και 40-42 ppt αλατότητα, νότιο Αιγαίο Πέλαγος) η συναγρίδα έφτασε στο εμπορεύσιμο βάρος (300-500 g) 14-18 μήνες μετά την εκκόλαψη των αυγών, μέσα στο εύρος των ήδη εκτρεφόμενων Μεσογειακών ειδών.

Η στοματική χορήγηση ανοσοδιεγερτών ( $\beta$ -1,3/1,6 γλυνάνες: MacroGard®, VitaStim®) για την προστασία των ψαριών έναντι των μεταδοτικών ασθενειών, αποδείχτηκε



ιδιαίτερα αποτελεσματική και παρά το ότι δεν εμπόδισε την εμφάνιση των ασθενειών, μείωσε σημαντικά την θνησιμότητα σε σχέση με τους μάρτυρες πληθυσμούς.

Η διατριβή περιγράφει την αλλομετρική αύξηση εννέα μορφομετρικών χαρακτηριστικών (ολικό μήκος, τυπικό μήκος, μεσουραίο μήκος, προεδρικό και μεταεδρικό μήκος, μήκος κεφαλής, ύψος σώματος, άνοιγμα στόματος, διάμετρος ματιού) σε ιχθύδια και ενήλικα άτομα (n=69-463). Καθόλη τη διάρκεια των πειραμάτων παρουσιάστηκαν επτά τύποι σκελετικών παραμορφώσεων, καθώς και ένας τύπος ανώμαλης ανάπτυξης των λεπιών. Επίσης περιγράφεται ένα περιστατικό "gas bubble disease" που προκλήθηκε από υπερκορεσμό του νερού εκτροφής με αέρια κατά 110%. Η εμφάνιση ενός περιστατικού "swimbladder stress syndrome" θεωρήθηκε ότι συνέβηκε κατά τη διάρκεια του εγκλιματισμού των άγριων γεννητόρων στις συνθήκες αιχμαλωσίας.

# 1 INTRODUCTION

## 1.1 Trends in aquaculture

World aquaculture production has more than doubled over the last decade and contributes today about 20 % to the total world aquatic production (FAO, 1995). While many of the natural stocks are exploited near or beyond their maximum sustainable yield, aquaculture production is expected to further increase in the future, but at slightly reduced rates, thus reaching a share of 25% of aquatic production by the year 2000 (Ratafia, 1995). Although the major part of this production is produced from herbivorous or omnivorous species (85%), which are relative low-valued, and produced mainly in extensive and semi-intensive farming systems, a trend directed towards the production of carnivorous, high-priced species is obvious, not only in most of the industrialized countries but also in several regions of the developing world. The rapid increase of aquaculture production in the category of fish such as seabass seabream groupers and snappers, which include especially high-priced species, demonstrates an impressive example of this general trend. Since the total production of all farmed finfish increased by 100% from 1984-1992, the seabass-seabream-grouper-snapper category increased at a remarkably higher rate (250%) during the same period. In 1992, the latter group represented nearly 6.5% of finfish aquaculture value, but only about 1% by volume (FAO, 1995).

The seabass and seabream farming in the Mediterranean area started about 15 years ago and - after an initial breathtaking development - seems to level out and struggles today with difficulties well known from other exponentially growing industries (Ridler, 1990; Ingle et al., 1990; Josupeit, 1995a; Nash, 1995). Production volumes had increased faster as consumer markets have been developing, which resulted in a considerable drop-off in prices (up to 40-50% since 1989, Josupeit, 1995a). These losses were only partly compensated by decreasing production costs per unit fish, going ahead with optimized production technologies and management strategies (Dendrinou & Thorpe, 1985; Tandler & Helps, 1985; Chatain-Guschemann, 1990; Colombo et al., 1990; Rosenthal, 1990). However, most of the industry had made initial investments when a rise in price was anticipated, causing severe pressure on financing schemes when prices actually dropped. Efforts to open new markets are on the way and necessary to stabilize demand driven prices in this fast growing branch of aquaculture (Josupeit, 1995b). Adequate environmental protection strategies have not yet been set up satisfactorily in most areas of the Mediterranean, which will probably cause problems in maintaining efficiency of production, particularly with regard to disease problems. Additionally, environmental protection measures which are presently under development and/or at the doorstep of being implemented, will increase expenses for monitoring and environmental protection programs. Therefore the industry is requiring further improvements in production efficiency to stay commercially viable. (Smith, 1991; Kissil & Lupatsch, 1992; Luzzana & Valfrè, 1993; Rosenthal, 1994; Giorgetti & Ceschia, 1994).

Future research priorities will focus on diversification of the production specter, including the development of new aquaculture species, alternative disease protection strategies and the optimization of farming technology and management.

## 1.2 Diversification of production

Diversification of the production in terms of species, types of products and processing modes or/and by employing several species into the production cycle of a farm, is an appropriate means to extend consumer markets. Companies producing a variety of products are less susceptible to price fluctuations, as only a part of their total selling will be influenced by sudden price drops of a single species or product. Additionally, they can shift production capacities, and thus reacting more flexible to changing market demands (Sweetman, 1992).

The sharp decline of wholesale prices for Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) forced producers of fry and round fish to considerably reduce production costs through improved husbandry and management to maintain their commercial viability. This has been achieved through a) improved rearing technologies, leading to higher survival and better growth rates as well as improved food conversion efficiency and b) through lean management and thereby improved utilization of facilities. A continuous year-round production of juveniles and market-sized fish, results in highly variable production costs, due to highly costly temperature control and extensive broodstock-management. However, continuous availability of fish would be highly desirable to open new markets. These operational obstacles could be diminished when producing a variety of different species, each matching seasonality of operation, when lowest production costs can be achieved.

The increase of production capacities within restricted areas, as currently observed in several coastal waters of Greece, is considerably contributing to the risk of disease transfer between farming locations. (Sweetman, 1992; Giorgetti & Ceschia, 1994; Paperna, 1984). As much as pathogens are species specific, the economic risk of epidemic disease outbreak in one species can be reduced by employing a multispecies production concept within one farm. This argument is even amplified, considering the strict limitations in the usage of antibiotics and other environmentally harmful therapeutants (Smith, 1991; Alderman et al., 1994; Rosenthal, 1994). Furthermore, this trend requires an increasing need to employ environmentally acceptable prophylactic agents such as vaccines or immunostimulants. It is within this context that the present study tries to emphasize the development of new means for preventive strategies. In this view, the following overview tends to highlight modern approaches which have been evaluated to formulate the concept of the present investigation.

## 1.3 Alternative disease protection strategies

Efforts in developing vaccines and new therapeutic agents are subsequent attempts to protect cultured fish stocks from undesired losses and place more emphasis on prophylaxis rather than treatment. Although a limited number of vaccines have so far been developed (Austin & Austin, 1987; Brinsinello et al., 1985; Lillehaug, 1989; Anderson, 1992), their application by bath or injection is still labour- and therefore cost-intensive.

A group of compounds, considered suitable for prophylactic application is characterized as  $\beta$ -1,3/1,6-linked glucans. These compounds are derived from yeast and fungal cell walls. They have recently gained considerable interest among scientists, feed producers and aquafarmers

(Onarheim, 1992; Raa et al., 1992; Roberson et al., 1994) because of their properties. Their stimulating effect on the non-specific immune response has been investigated in a series of studies, involving important aquaculture species, such as the common carp (*Cyprinus carpio*), the Atlantic salmon (*Salmo salar*) and coho salmon (*Oncorhynchus kisutch*), the channel catfish (*Ictalurus punctatus*) and the yellowtail (*Seriola quinqueradiata*) (Yano et al., 1989; Robertsen et al., 1990; Chen and Ainsworth, 1992; Nikl et al., 1993; Song & Hsieh, 1994). Most of these studies employed glucan preparations through intra-peritoneal injection, but several recent investigations showed also the possibility of oral administration of these substances in physiologically effective concentrations (Siwicki et al., 1993; Nikl et al., 1993; Yoshida et al., 1995; Efthimiou, 1996). The stimulation of the non-specific defense response has been related to significantly reduced mortality rates after challenge with various pathogens, which cause high losses among cultured species (*Aeromonas* sp., *Vibrio* spec. *Streptococcus* spec, etc.). The ease of application via the oral route, their potential ability to protect animals against many different pathogens with a single treatment, and their complete environmental harmlessness makes the prophylactic use of these therapeutics extremely interesting for the aquaculture industry.

However, further work on the adequate dose and duration of application as well as the interactions of their metabolic effects in relation to various stress responses under commercial culture conditions is needed to allow a reliable evaluation of the successful application strategy of  $\beta$ -glucans in aquaculture.

#### 1.4 Aquaculture candidates for the Mediterranean aquaculture

Presently several marine fish species are considered as potential aquaculture candidates in the Mediterranean area. Among them, sparids like the red porgy (*Pagrus pagrus*), the black seabream (*Spondyliosoma cantharus*), the sheephead bream (*Puntazzo puntazzo*), the common dentex (*Dentex dentex*), as well as members of the family Serranidae like several groupers (*Epinephelus guaza*, *E. aeneus*, *E. alexandrinus*) and the Mediterranean amberjack (*Seriola dumerilii*) (Faranda et al., 1985; Sweetman, 1992; Divanach et al., 1993; Bibiloni et al., 1993; Berry, 1994; Efthimiou et al., 1994; Marino et al., 1995; Wray, 1996; White, 1996). A high growth potential and a lucrative market price are the basic criteria in the preliminary selection process. However, almost no basic knowledge of the life requirements of these species, as well as the specific bio-technological needs for a successful culture are available yet. The knowledge gap becomes obvious when comparing the number of publications for the period 1988-1995 (ASFA, 1995: CD-ROM search), on the above stated aquaculture candidates with those candidates for cold water aquaculture such as the Atlantic halibut (*Hippoglossus hippoglossus*) or the Atlantic cod (*Gadus morhua*) during the same period. While for the former species grouping only 22 papers occurred, the research output for the latter group reached 122 for halibut and 267 for cod.

Problems with artificial reproduction of "new species" are manifold. The reliable supply of large quantities of fertilized gametes is reported to be difficult to achieve in the red porgy, black seabream, the groupers and the amberjack. For example in tropical groupers, it has only recently been identified that an intensive hormonal treatment, well tuned to the moon phases is

necessary to induce spawning (Ruangpaint, 1993), while black seabream are benthic spawners, laying adhesive eggs (Camus and Besseau, 1986), and therefore require different methods for egg collection and handling than other closely related species. It is surprising that almost no information on their natural spawning grounds is available in order to quickly learn about biological and biotechnological requirements for spawning. Furthermore little is known on breeding behaviour of these species, so that brood stock handling is still based on trial and error. Mediterranean amberjacks have never been shown to release sexual products in captivity (Marino et al., 1995).

First feeding of larvae on natural food chains is extremely difficult, especially in species where the size of the mouth is very small, as has been reported for most groupers (Ruangpaint, 1993) and for common dentex (Franicevic, 1991; Glamuzina et al., 1989). Compared to the already successfully cultured marine species such as the gilthead seabream (*Sparus aurata*) or the European seabass (*Dicentrarchus labrax*) the initial feeding problem adds to the overall difficulty in developing proper culture technology. Thus, only the expensive supply with especially small-sized zooplankton can solve this problem in some cases (James & Abu-Rezeq, 1989; Snell and Carillo, 1984.). A high sensitivity against mechanical stress during early larval rearing of groupers and common dentex has also been noted, and this makes handling procedures such as transfer and grading very difficult. Additionally, these species develop a strong agonistic behavior and thus cannibalism can cause high losses among larvae and juveniles (Franicevic, 1991; Efthimiou et al., 1994; Smith and Reay, 1991). The lack of suitable complete diets caused nutritional imbalances during all on-growing phases, when diets of established culture species were administered in first rearing trials with new species (Bromley & Sykes, 1985; Watanabe & Sakamoto, 1992; Brown, 1995; Tibaldi et al., 1996). Nutritional imbalances are often associated with low and highly variable survival rates, sub-optimal growth rates, a low food conversion efficiency, malformations and an increased disease susceptibility (Richly & Spannhof, 1979; Crampton, 1985; Hilton, 1989; Landolt, 1989; Koven et al., 1990; Tacon, 1992; Takeuchi et al., 1992).

This short summary of the problems in the culture of new species makes clear that, further work is needed, defining species specific life-requirement-preferences, to fill the wide gap between a relative low level of biological knowledge concerning possible aquaculture candidates, and the high interest of the aquaculture industry in product diversification. Besides the technical feasibility of culture, environmental and market oriented aspects play a major role too in the evaluation process for the selection of the most suitable aquaculture species.

## 1.5 Objectives of the presented study

In light of the elaboration presented under 1.1 to 1.4. the objectives of the presented study have been determined as follows:

- The species selected should be one of high commercial importance to the Mediterranean area, with related species of importance in other warm water regions. The choice has been set for the common dentex (*Dentex dentex*).

- *Identification of biological traits important for growth and feed conversion*

Little information is available on growth, survival and food conversion efficiency of this

species. Therefore, the experiments conducted during this study should provide a sound data base for the evaluation of the commercial value of common dentex, regarding its suitability for aquaculture purposes. Since feed costs do account for about 50% of the whole production costs in intensive farming systems, and will probably still increase due to limited resources of cheap animal protein (Tacon, 1995), growth performance and food conversion are of special interest for the economic evaluation of a potential aquaculture candidate. Therefore, growth rates, food consumption and conversion efficiency during different life stages and under various nutritional regimes were thoroughly estimated in several experiments to provide information for decision making processes.

- *Testing culture conditions known to be suitable for established aquaculture species, for which requirements are considered to be similar*

Little information is available on culture technology, behaviour and its implication on disease susceptibility and mortality. High mortalities, due to an increased disease susceptibility or cannibalism can drastically reduce the suitability of an aquaculture candidate. Therefore, a regular monitoring of agonistic behaviour and mortalities through all experiments was conducted to provide detailed information on mortality causing factors.

- *Description of morphometric development and its biological implications for cultivation*

Little information is available on morphometric characteristics of this species in response to culture technology. Therefore, 9 morphometric characters measured in about 600 live specimens with a size range of 12.7 to 470 mm, and analyzed to provide an extensive database for the identification of allometric growth patterns, an important tool for the understanding of behavioural and physiological changes during the life cycle of a species and in response to environmental stress (Fuiman, 1983; Fukuhara, 1992; Otterå & Folkvord, 1993; Osse & Boogart, 1995).

- *Behavioural aspects related to the development of cannibalism*

Little is known on the behavioural development of this species held under culture conditions. The occurrence and severity of agonistic behaviour and/or cannibalism at different life stages were, therefore, determined by regular counts, and by investigating of affected specimens. Possible relationships between cannibalism and nutritional status or size distribution of fish within the culture were identified and described, to see whether such relationships could be used to quantify the effects.

- *Testing of methods to reduce stress induced disease susceptibility*

Nothing is known in relation to handling stress and disease susceptibility of the species when reared at high density under commercial culture conditions. The regular outbreak of epidemic diseases, when fish were intensively stressed by handling, were studied to stress the need for an environmentally acceptable and effective prophylactic therapeutant. As a first attempt to develop stress investigating strategies, the application of two different immunostimulants in the culture of common dentex was investigated. The experiments aimed to provide information on the effect of orally administered  $\beta$ -1,3/1,6 glucans with regard to growth performance, mortalities and parameter of the non-specific immune system in a sparid fish such as the common dentex.

# 2 THE GENUS DENTEX

## 2.1 Biology and distribution

Order: Percoidei (Perciformes)  
Family: Sparidae  
Genus: Dentex

The genus *Dentex* comprises 8 species (*Dentex angolensis*, *D. canariensis*, *D. congoensis*, *D. dentex*, *D. gibbosus*, *D. macrophthalmus*, *D.maroccanus*, *D. tumifrons*) which are all distributed within the Mediterranean-Black-Sea area and in the central Eastern Atlantic. *Dentex kokeni* is known only from palaeontological studies on fish otoliths found in The Netherlands.

**The common dentex (*Dentex dentex*, Linnaeus, 1758)**  
Synonyms: *Sparus dentex*, L., 1758), *Dentex vulgaris*, Valenciennes, 1830; *Dentex vulgaris*, Günther, 1859)

The common dentex, (*Dentex dentex*, Linnaeus 1758), is a sparid fish who inhabits the Mediterranean, most commonly south of 40", rarely in the Black Sea and the Atlantic from Bay of Biscaya to Cape Blanc and Madeira, exceptionally to the British Isles (Bauchot & Hureau, 1986). He lives in inshore waters on rocky bottoms to 200 m, but more common between 15 and 50 m (Bauchot & Hureau, 1986). Juveniles building small schools whereas old specimens live solitary. The common dentex is a strict carnivore fish through all live-stages, which is indicated by the early development of the strong canine shaped teeth, and the short intestinal tract. The impressive teeth of these fish have been also the origin of the genus name (lat.: dentis = tooth, peak). With a maximal length of about 1m, the common dentex is one of the largest species among the Sparidae. It shows an extended reproduction period from the end of April till the beginning of July in the Mediterranean Sea. Although some authors reported this species as a protandrous hermaphrodite (Glamuzina et al., 1989), a more recent study showed

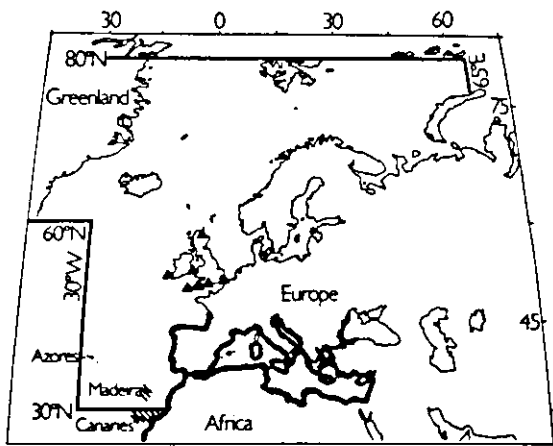


Figure 1. Distribution map of common dentex (*Dentex dentex*) after Bauchot & Hureau, 1986.

that this species is probably gonochoric with only exceptionally cases of sex reversal. Males appear intense bright blue coloured, while females are more pallid and emerald green (Pastor et al., 1995).

Recently, first results on fecundity, egg and larval development have been based on preliminary rearing experiments (Glamuzina et al., 1989; Franicevic, 1991; Kentouri et al., 1992; Divanach et al., 1992; Pastor et al., 1995). Spawning is reported to take place at temperatures between 15°C (Bibilioni et al., 1993; Pastor et al., 1995) and 21-22 °C (Glamuzina et al., 1989). Captive genitors release their sexual products either spontaneously (Pastor et al., 1995; own observation), or after hormonal treatment (Glamuzina, 1989, Franicevic, 1991) into the water. The common dentex is a partial spawner, and one female can release several Mio's of eggs per spawning season. Maturity is first observed in fish above 500 g (Glamuzina, 1989), at the end of second year. Pastor et al.(1995) found that two years old fish have already mature gonads and are either male (71.2%) or female (28.8%).

Fertilized eggs are spherical in shape, transparent and about 0.983 - 1.027 mm in diameter with a single oil droplet of  $0.254 \pm 0.008$  mm, positioned at the periphery of the chorion (Kentouri et al., 1992). Eggs are buoyant in seawater, not-adherent and can be easily collected from the watersurface. Franicevic (1991) counted 1450 eggs per gram of egg mass. Embryonic development takes 42 - 80 hours from fertilization until all larvae are hatched at ambient conditions of 18.5 - 17 °C (Glamuzina et al., 1989; Kentouri et al., 1992; Pastor et al., 1995). The newly hatched larvae are 2.17-2.61 mm in total length (Glamuzina et al., 1989, Divanach et al., 1992; Pastor et al., 1995). Feeding starts 3 to 4 days after hatching when larvae reach a size of 3.4 - 3.6 mm TL at 19 - 18 °C incubation temperature (Divanach et al., 1992; Pastor et al., 1995). Metamorphosis and agonistic behaviour starts much earlier in common dentex than in seabass and seabream larvae (Koumoundouros pers. communication). High mortalities during intensive larval rearing have been associated with inadequate size of life food at first feeding (Glamuzina et al., 1989; Franicevic, 1991), general poor culture conditions and nutritional deficiencies (Pastor et al., 1995), strong cannibalism (Franicevic, 1991) and microbial infections (Pastor et al., 1995).

Results on rearing of postlarvae and juveniles are even more seldom compared to the preliminary studies on reproduction, embryonic and larval development. Postlarvae reached 31 mm total length in 55 days after hatching at ambient temperature (17 - 23 °C), while survival rate was 0.9% only (Pastor et al., 1995), and raising of 3 g fry in 90 days from hatching has recently been reported by Bibiloni et al. (1993) at survival rates of 2%. A total length of 270 mm was reported from 13 month old specimens fished in the Gulf of Neapel (Lo Bianco, 1909).

## 2.2 Fisheries and Catches

The total catch of the common dentex fluctuated between 5404 metric tones (mt) in 1984 and 7277 mt 1993, with increasing catches from 1990 on (Table 1). In comparison to other dentex species the common dentex was the most important species in fisheries in 1994 and made up 28% of the total catch of this genus. The exploitation of largeeye Dentex (*D. macrophthalmus*),



Table 1: Total catch of Dentex species during 1984 - 1993 in metric tonnes (FAO, 1995). D. spp. includes all catches of the genus Dentex, which are not classified on the species level.

	1984	87	88	89	90	91	92	93
D. dentex	5404	5875	5583	5791	8681	7664	10327	7277
D. macrophthalmus	13280	10457	8208	9239	6205	6140	2050	1914
D. angolensis	56	224	758	853	640	1632	2377	428
D. congocensis	—	—	111	36	180	98	151	364
D. spp.	7584	9045	17804	14238	11250	12472	14378	16369

many years the most important dentex species in fisheries, decreased continuously from 13280 mt in 1984 to 1914 mt in 1990. Terre (1980) found the catch levels during 7 years between 1965 and 1978 considerably higher than those derived from the MSY calculation. This could be one reason for the continuous decline of the landings of this species. The missing landings of largeye dentex seem to increase fishery effort on other Dentex species such as *D. dentex* and *D. angolensis*. The development of catches in *D. angolensis* indicates already a similar overfishing (Table 1).

Highest landings of common dentex in 1993, with a total of 4239 mt are reported from the eastern central Atlantic (Fishing area 34). Here, Italy with 80% is by far the major fishing nation followed by Greece which contributes with 18.4 % (770 mt) to the landings in this area. In the Mediterranean and Black Sea (Fishing area 37) the distribution of landings between nations gives a similar picture. The total of 2996 mt fished in this area is shared between Italy (75%), Turkey (7.6%), Greece (7.6%) and Tunisia (7.1%). During the last years a shift of catches from the Mediterranean Sea to the eastern central Atlantic took place, especially by Italy. The increase in total landings of this species is therefore not achieved by a more intensive exploitation of the Mediterranean population, where landings are decreasing, but through the increase of fishing efforts in new fishing grounds.

## 3 MATERIALS AND METHODS

### 3.1 Experimental site and facilities

All experiments were carried out at the aquaculture facilities of the Institute of Marine Biology of Crete, which are located at the exit of the harbour of Iraklion (Crete; Greece). The installed water supply system consisted of a shore-based pumping house, a header tank for degassing, which also allowed to feed the supply lines with a constant water pressure. A variety of different types (sizes, shapes) of fish tanks were also available. The supplied sea water passes by gravity through a rock-gravel-sand bed of several meters depth before entering the sump. In this way a coarse pre-filtration is achieved. Due to the high water flow rates (200 - 300 m<sup>3</sup>/h) through the system (compared to the size of the header tank: 10 m<sup>3</sup>), retention time in this tank was relatively short (within the range of 2-3 minutes). From the header tank, water was flowing by gravity to the different tanks of the experimental culture system.

### 3.2 Experimental Periods (Objectives and design implications)

The experimental scheduling was developed step by step, oriented on experiences gained during rearing, screening trials and subsequently conducted investigations. This rather uncommon strategy was necessary, as rearing success would have been endangered, if a priori, a fixed experimental lay-out had been applied to an almost entirely unknown fish species. The type of experiment or rearing phase, date, duration, initial and final number as well as the size range of fish investigated or reared are summarized for the entire study in Table 2.

The experiments of this study were conducted during a period lasting from August 1992 until September 1994. The following chapter summarize the time course of experimental planning, as well as the main objectives of the investigations carried out during this study.

The experimental concept and argument leading to design adjustments are presented in the Materials and Methods section as explanatory guideline. This seems to be justified because very little experience is available for this new candidate. To separate this argument from the methodological parts this text is given in italics.

#### 3.2.1 Objectives and experimental schedules in 1992: Experimental periods (EP) 1 & 2

*A first screening period of 9 weeks (EP 1, Table 2) was arranged to get basic information on social behaviour, feed preferences, activity pattern of fish as well as first results on growth performance and mortalities. These first observations and preliminary results should serve as a base for the development of relevant scientific questions and allow the reliable scheduling of experiments to prove these hypothesis. This period was also necessary to provide a training in handling practices of this unknown species, which is a necessary and often underestimated aspect in developing reliable rearing experiments.*

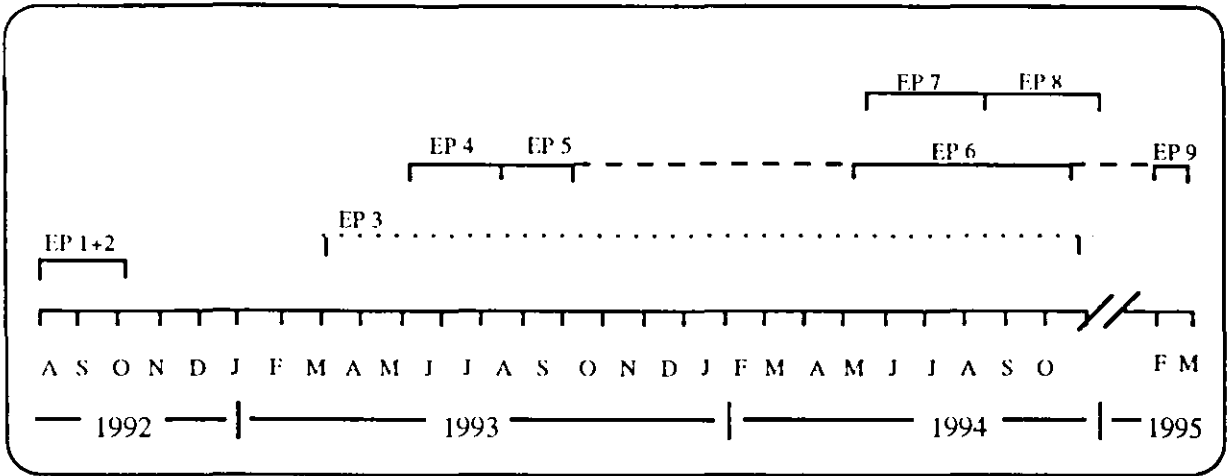
In August 1992, a total of 506 juveniles, distributed in three size classes (0.18 g, 1.71 g and 3.7 g) were kept at the facilities of the Institute of Marine Biology of Crete. These fish originated from semi-intensive rearing trials ( $\Rightarrow$  3.4). Eggs were obtained from captive broodstock fish

**Table 2:** Type of experiments, date, duration, initial and final number (N) and the size range of fish investigated or reared are summarized. The kind of lay-out (single (S), duplicate (D), triplicate (T)) describes the number (n = 1,2,3) of parallel groups receiving the same treatment. The chronological sequence of the experimental schedule ( $\Rightarrow$  Figure 2) was mainly determined by the natural spawning period of the common dentex in the southeast Mediterranean Sea (April and June). The number of the experimental period (EP) will be referred to in the text.

EP No.	Type of experiment or rearing phase (Single, Duplicate, Triplicate lay-out)	Dates (start-end)	Duration (days)	N (start-end)	Size ranges
1	Screening trial: handling, behaviour, food acceptance (S-3 size classes)	31.7 - 25.8.92	25	605-210	0.81 g - 8.87 g
2a/b	Preliminary results on growth performance and disease susceptibility (T-3 size classes)	26.8. - 5.10.92	40	180-120	3.73 g - 28 g
3	Broodstock handling and transfer (S)	10.3 - 12.3.93	2	8	1000 - 2500 g
4	Larval rearing under semi-intensive conditions (S)	4.5 - 6.6.94	33	40.000-2000	3.47 mm - 13 mm
5	Growth performance and agonistic behaviour related to dry and moist diets (T)	25.7 - 10.9.93	46	900-600	2.4 g - 31.3 g
6	Growth performance of 11- 14 months old fish; comparison of different diet moisture level (D)	11.4. - 13.7.94	91	350-320	98 g - 282 g
7*	Egg incubation, larval rearing and weaning (D)	24.4 - 7.7.94	75	250.000 -5000	
8	Effects of non specific immuno-stimulation on growth ,mortality and disease resistance (T)	8.7 - 28.9.94	51	1980 - 1280	2.4 g - 46.9 g
9	Transfer of 21 months old dentex from Iraklion to a bream-bass hatchery (Vasni), Gas bubble disease (S)	2.2 - 7.2.95	5	30	700g - 1500 g

\* The second larval rearing period (No 7) was mainly supporting the experimental setup of the Ph.D. theses of Mr. G. Koumoundouros.

in May of the same year during the natural spawning period. Most of these juveniles expressed strong skeletal deformations ( $\Rightarrow$  3.18), and the commercial pellets offered by automatic feeder systems were poorly accepted. *During tank cleaning, many fish expressed a highly nervous behaviour, such as burst swimming movements and jumping out of the water, thus indicating poor rearing conditions. High mortality rates (up to 5 % per day) were recorded for all three size classes. The poor condition of fish described above, was suggested to be related to low food acceptance, a nutritional imbalance of the administered food, and/or an inadequate feeding regime.* Therefore, a screening period (EP 1), testing a variety of fresh food products similar to the natural diet of dentex was conducted to (a) identify preferences in type, size and palatability of different foodstuffs and (b) stabilize the critical health condition of these



**Figure 2.** Time schedule for experimental periods (EP) of this study (see Table 2). Broken lines indicate periods of maintenance; dotted line indicate broodstock management. Lines on the same level represent fishes of common origin (larval rearing).

juveniles. After changing from dry to fresh and moist diets, administered by frequent hand-feeding, fish condition had increased considerably after about 3-4 weeks. Mortality rates were strongly reduced ( $< 1\%$  /day), nervous behaviour less pronounced, and fish fed actively on a prepared moist diet. At this moment a preliminary experiment was set up to compare the performance of a moist diet with a commercial seabream pellet (EP 2a). For this reason, fish of each size class ( $n = 3$ ) were distributed equally into two tanks. Subsequently 3 test groups (Size 1-3) were fed either the moist diet or the commercial seabream diet.

Since almost nothing was known about nutritional requirements of this species, a moist diet which was prepared to be similar to the natural fish diet of the carnivore dentex and supplemented with vitamins and highly saturated fatty acids in amounts sufficient for most marine fish species (Howell, 1984; Obach et al, 1992; Takeuchi et al., 1992), was thought to provide a suitable reference diet, indicating optimal nutritional conditions. Commercial seabream diets have been proven already to provide a potential diet for a variety of breams (Divanach et al., 1992); they are available year-round, easy to feed and store. Therefore, a high-quality pellet (Ecostart 17  $\Rightarrow$  Table 6) was chosen as second test diet for this experiment.

Each of the two test diets were fed to tanks containing fish of each size class ( $n = 3$ ), respectively. Food consumption was recorded for individual feedings (Table 6) and growth was followed by weekly measurement of individual fish. After a period of two weeks feeding on commercial pellets to all tanks, sudden mortalities occurred due to an infectious disease ( $\Rightarrow$  3.16, disease recognition and treatment). None of the fish in the parallel group, which was fed on moist pellets acquired the disease during this period.

Due to the severe disease, fish were in such a poor condition that no further weight measurements could be conducted. Therefore this experiment was stopped and after a 10 days observation period, the 3 groups of healthy fish, feeding on moist pellets, were re-distributed into 4 tanks (2 size classes) and the feeding trial (comparing moist against dry pellets) was repeated (EP 2b). Unfortunately this experimental set-up could also not provide reliable results, since several fish were missing in the dry pellet group, most probably predated during the night by a cat. Therefore, after another 3 weeks this experiment was terminated.

### 3.2.2 Objectives and experimental schedules in 1993. Experimental periods 4 & 5

Based on the 1992 rearing experience, the objectives for the 1993 experiments aimed on the determination of reliable data on growth performance of juveniles under favourable rearing conditions. The experiment of 1992, comparing a commercial dry pellet with a self-prepared moist pellet was repeated in triplicate lay-out with juveniles reared during spring and summer 1993 under semi-extensive conditions. Due to the favourable larval rearing conditions, these fish did not express an increased proportion of malformed specimens and the group was considered as good quality fish to start this series

In comparison to the 1992 experiments, growth was still determined in weekly intervals. However, weighing stress was minimized by taking only small random samples of fish (10% of each tank population), instead of weighing all fishes as has been done in 1992. The excessive weighing stress in 1992 was thought to be one of the reasons for the disease outbreak. Since the experimental tanks were relative small in diameter (105 cm), handling was easy and all parts of the tank were accessible during netting. Therefore, size selectivity within the sub-samples was thought to be negligible. Similar to the previous year, individual fish were measured rather than groups to obtain information on the development of the size distribution within the tank population, an important factor especially when dealing with the agonistic behaviour of juveniles. All sampled fish were photographed and 9 morphometric characters were measured (3.4  $\Rightarrow$  morphometric measurements). This was done to obtain a sound database on the growth pattern of this species as well as to identify differences in growth performance between the two test diets. Treatment schedules for infected fish were established during this experimental period (3.16  $\Rightarrow$  disease recognition and treatment).

### 3.2.3 Objectives and experimental schedules in 1994. Experimental periods 6 - 8

Differences in growth performance, disease susceptibility and agonistic behaviour between fish groups that had been fed on moist or dry diets became obvious during the experiments conducted in 1992 and 1993.

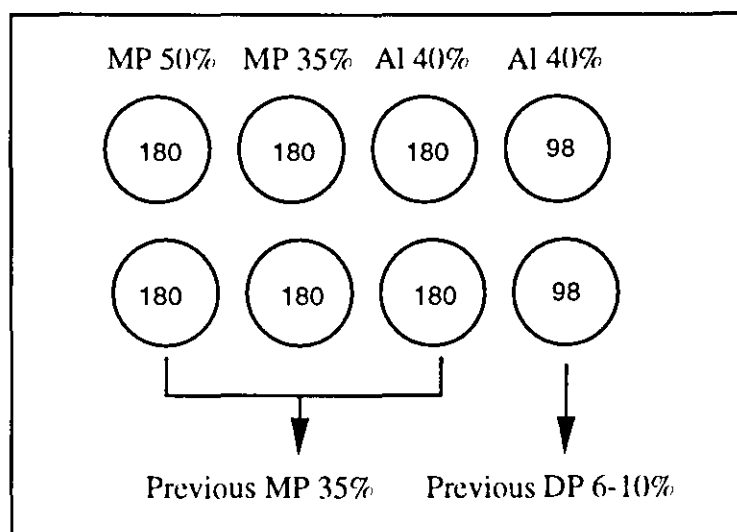
*Since the crude composition and the energy content of the test diets did not differ considerably (Table 6), the moisture content was assumed to be the influencing factor, which in turn affected food acceptance, palatability, appetite and digestibility in the common dentex. This hypothesis was supported by results of Jobling (1986, 1987), who found that the digestibility coefficients of formulated feeds were 5-10% lower than those of natural food organisms. The author suggested that an over-rapidly emptying of high energy, small particles from the stomach could lead to an overloading of the intestinal digestion capacity and a reduction in adsorption efficiency.*

The objective of the study, therefore, was to answer the question whether the different moisture content of the test diets could be related to the differences in growth performance between moist and dry diets observed in earlier experiments (EP 2 & 5). Additionally, this experiment should provide a data base to establish for the first time the growth performance of ongrowing common dentex (age: 1+ group) under favourable culture conditions. Thus continuing the intensive growth study of the early juveniles (age 0+ group) which had been carried out a year ago (EP 5).

### 3.2.3.1 Influence of feed moisture content on growth performance in age 1+ dentex (Experimental Period, EP 6); Experimental set-up:

Origin of experimental fish: The juvenile dentex reared in 1993 (EP 4 & 5) were kept through the winter respectively to the feed - moist or dry pellet - applied previously in separate tanks. They were continued to feed either a moist diet (composition varied due to availability of fresh fish) or a bream grower pellet (Aqualim 2, Table 6) until spring 1994 (Figure 2). In March 1994 these both groups expressed strongly different mean weights for the dry pellet fed (98 g) and moist diet fed (180 g) fish. Due to the considerably higher mortality in fish fed dry pellets, only 108 fish compared to 250 fish fed the moist diets had survived the first year of rearing.

Stocking and feeding schedule: Six tanks were stocked with 40 of the larger fish and two tanks with 54 of the smaller size group, respectively. A commercial dry pellet (Aqualim 2, Table 6) was prepared with a similar moisture content as the moist pellet (40%) and fed to the previous dry pellet fish and also to two tanks of the previous moist diet fish. Two tanks were continued to feed on the "normal" moist diet (35% moisture) and another two tanks were fed the moist pellet containing an elevated moisture content (50%) (Figure 3). This experimental arrangement should (a) clear the question if fish expressing a restricted growth performance while fed with a commercial dry diet could improve diet utilization when the same diet is fed with a considerable increased moisture content, (b) allow to compare the growth performance of fish changing the diet from the self-prepared moist pellet to a commercial bream grower pellet with similar moisture content (40%), (c) show if the elevation of the moisture content of the moist diet up to 50% does further influence food utilization in common dentex and (d) give results on growth performance, food conversion efficiency and mortalities in one year old dentex fed a well defined moist diet prepared at site and reared under intensive conditions.



**Figure 3.** Experimental lay-out EP 6 (Influence of diet moisture content on growth performance). MP = Moist Pellet, Al = Aqualim (commercial grower pellet), percentage (%) = moisture of feed; 180, 98 = initial individual weight (g); Previous = main food quality until the experiment started. Composition and crude analysis of both tests diets are given in Table 6.

The feeding trial lasted for a period of 13 weeks, during which growth was determined in 3 weeks intervals, weighing all fish in groups of 3-6 specimens. Food consumption was recorded for individual feedings (2-3 per day).

### **3.2.3.2 Effects of immunostimulants on growth performance, mortalities and non-specific defense mechanisms in juvenile dentex (Experimental period, EP 8).**

*During the first two years of rearing of common dentex a high disease susceptibility was observed. Stress induced by rearing conditions, the high experimental handling stress as well as nutritional imbalances were assumed to weaken the immune system of this species. The relationship between stress, nutrition and the immune response has been extensively reported from other fish species (Landolt, 1989; Anderson, 1990; Salonijs and Iwama, 1993).*

*The stimulation of the non-specific immune system by  $\beta$ -glucans has recently attained considerable attention among fisheries scientists as well as aquaculturists. It was suggested that these substances could provide a possible tool for the successful culture of common dentex, while protecting fish during stressful handling periods from infectious disease. Thus, an experiment was planned to estimate for the first time the effects of orally applied immunostimulants on growth performance, mortalities and non-specific defense mechanisms. Since the knowledge of oral application of immunostimulants in fish was very poor at the time of planning this experiment (one study by Raa et al., 1992) and nothing was known on the effects of long term application on growth performance or natural mortalities in fish, this investigation should provide also basic information on the application of immunostimulants in fish. The control group of this experiment should also provide data on growth performance of common dentex juveniles under high stocking densities and optimized rearing conditions (grading, adjusted current speed) compared to the rearing experiment of the previous year (EP 5).*

#### **Experimental set-up:**

**Origin of experimental fish:** To attain a sufficient number of test fish (2000-3000 juveniles), a larval rearing program was set up in May 1994. The larval rearing was conducted in collaboration with Mr. G. Koumounduros. Results of the larval rearing mainly supported his Ph.D. theses of common dentex embryonic and larval development and the establishment of quality criteria. The fry produced during this period (5000 pieces of 1g) provided the experimental fish for the experiment on immunostimulation (EP 8).

**Stocking and feeding schedule:** At the beginning of the experiment, fish were sorted into two size groups, small (2.4 - 2.5 g) and large (4.09 - 4.55 g), a necessary procedure to reduce agonistic behaviour. A total of nine tanks were each stocked with 220 fish; three tanks with small, and six tanks with large fish. Three replicates were run for each of the two glucans and for the control group. The replicates of each test group consisted of 1 tank with small fish and 2 tanks with large fish.

Juvenile common dentex were fed moist diets over two months. The 3 test groups received diets supplemented every second week with one of the two tested immunostimulants, MakroGard or VitaStim, or a diet without supplement (control). All fish were weighed at weekly intervals to a) obtain data on growth and feed conversion and b) generate repeatedly a defined stress situation to amplify the possible effects of the immunostimulant treatments on growth, mortality and nonspecific defense mechanisms.

Mortalities during the experiment were recorded as described under 3.17. At the end of the experiment, blood-samples were taken and analyzed to define the status of the non-specific immune system (3.19  $\Rightarrow$  blood parameter). A final sample of 40 fishes was randomly sampled at the end of the experiment from each of the test tanks. The weight distribution, external visible damages and anomalies were recorded.

### 3.3 Experimental conditions

The experimental conditions during the main experimental periods are summarized in Table 3. The water exchange rates were chosen in order to provide favourable water quality. A regular adjustment of water exchange rates to the increasing biomass load (stocking density), due to the fast growing fishes, was necessary to maintain good water quality throughout the entire period. Only during experimental period 5, when the effects of feeding isocaloric moist and dry diets were investigated, the high initial water exchange of 100 % / h was kept constant throughout the experimental period.

At intensive stocking densities of up to 14.4 kg/m<sup>3</sup> (EP 8) water quality still remained within favourable levels. The determined concentrations of total ammonia (NH<sub>4</sub><sup>+</sup> + NH<sub>3</sub>) reached maximum values of 0.224 mg/l, which equals an unionized ammonia concentration of 0.009 mg/l NH<sub>3</sub> at pH 8 and a temperature of 25 °C (EIFAC, 1986), far below critical values estimated for other marine fish species (Sadler, 1981; Wickins, 1981; Meade, 1985; Handy & Poxton, 1993). During most measurements nitrite was not present in the samples and even highest concentrations reached only low values of 0.002 mg/l which is also far below levels

**Table 3:** Experimental conditions monitored at a daily (temperature, pH, oxygen) or weekly basis (salinity, total ammonia, nitrite, total suspended solids) during different experimental periods (EP 1-8). Minimum and maximum values are given for each parameter. The theoretical water exchange rate (%/h) is given as water flow (l/h) / tank water volume (l) \* 100. Stocking density was determined in regular intervals as biomass increase and is given as minimum and maximum values.

	EP 1 +2	EP 5	EP 6	EP 8
Temperature °C	22.8 - 25.7	24.4 - 26.2	15.8 - 25.0	24.9 - 25.8
Oxygen (mg/l)	5.4 - 6.6	4.8 - 5.9	3.5 - 5.0	4.0 - 5.2
pH	8.21 - 8.48	7.71 - 7.80	7.11 - 8.04	7.80 - 8.02
Salinity (ppt)	39 - 41	41 - 42	40 - 42	40 - 42
Total ammonia NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup> (mg/l)	-	-	0.056 - 0.224	0.050 - 0.210
Nitrite (NO <sub>2</sub> -) (mg/l)	-	-	0.000 - 0.003	0.000 - 0.002
Total Suspended Solids (TSS) (mg/l)	-	-	37.63 - 46.90	-
Theoretical water exchange rate (% tank volume / hour)	50 - 100	100	70 - 100	100 - 180
Stocking density (kg/m <sup>3</sup> )	0.36 - 2.95	0.91 - 2.62	3.98 - 9.44	1.12 - 14.40



which are known to cause negative effects on growth in other fish species (Kawamoto, 1961; Saroglia et al., 1981; Handy & Poxton, 1993). The relative high salinity of sea water additionally reduces the toxicity of unionized ammonia and particularly that of nitrite. Thus, even several times higher values (daily peaks) for these water quality factors, which have been observed during daily fluctuations in intensive rearing systems (Rosenthal et al., 1979; EIFAC, 1986) should not have reached growth-affecting levels for fish in general.

The concentration of Total Suspended Solids (TSS) was determined only during the experimental period on the effect of diet water content on growth performance (EP 6). However, due to the similar types of feed and rearing conditions (water exchange rate, stocking density, tank design), which were employed during the other experimental periods, TSS should have been accumulated at similar level. Although information on the negative influence of TSS on fish health and growth in culture systems is limited and mainly related to salmonids, the here observed levels of maximal 49.8 mg/l should not have affected fish health and growth (Wedemeyer, 1981; Wickins, 1981; Redding et al., 1987). However, there are very few studies investigating long term effects of elevated suspended solid concentrations (Efthimiou, 1992), and nothing is known on critical levels in sparids such as the common dentex.

During experiment 6 (EP 6), oxygen concentrations were accidentally reduced to values of 3.5 mg/l for a 3 days period (week 6) because the water supply line was disconnected from the header tank and fish tanks were fed with relative low oxygen water (45-50% saturation), pumped directly from the sump. During this experimental period a notable increase of ambient water temperature (16.1 - 25°C, Figure 23) due to seasonal fluctuations was evident; the other experimental periods were carried out during the summer months only, during which temperature remained relatively constant.

### 3.4 Egg incubation and larval rearing techniques

Fertilized eggs were supplied from wild broodstock fish kept in commercial fish farms during the natural spawning period (April-May). Eggs were collected at the 2-8 cell stage and transported in plastic bags (100,000-200,00 eggs per bag), filled with 5-8 l seawater and 10-15 l pure oxygen. Spawning took place at 16-18 °C and temperature commonly had risen during transport up to 20-22 °C. Incubation took place in 500 L circular tanks with conical shaped bottom at densities of 50-150 eggs/l. At a mean temperature of 17 °C, hatching took place at 40-50 hours after fertilization.

Hatched larvae were transferred into circular tanks of 40 m<sup>3</sup> water volume, here referred to as Mesocosmos (Øiestad, 1984; Divanach & Kentouri, 1983). These had been filled with filtered sea water 10-14 days prior to the stocking of the larvae, to allow a rich zooplankton bloom to develop, providing the initial food source of the fish larvae. When larvae had already developed (7-10 days after hatching), rotifers (*Brachionus plicatilis*) and artemia nauplii (*Artemia salina*) were supplemented to enrich the limited natural plankton source of the Mesocosms. After about 2 weeks, weaning on small pieces of minced fish filet started and after 3 weeks of incubation the juveniles were harvested, either by the use of a large enclosure net (1993, 1994)

or with a siphon technique (1994), where the juveniles were attracted by a strong light to swim into a submersed plastic cone which was connected via a siphon with the terminal culture tank.

Cylindroconical tanks were used for further rearing of juveniles in 1994 (EP 7). Initially these tanks were filled with the Mesocosms water, containing a dense algae bloom and natural occurring zooplankton. This water was subsequently exchanged by filtered fresh sea-water during the first 48 hours after transfer of the larvae. The following 2-3 weeks period, water exchange rates increased from initially 5-10 % up to 50% of tank volume per hour, respectively to increasing biomass and feed ration.

### 3.5 Origin, maintenance and transport of broodstock

Wild brood fish of 1-5 kg mean weight were collected from fishermen, stocked in a floating net cage (5x 5 x 6 m) at the island of Leros and maintained as brood stock. In this facility dentex were feeding actively on a diet of various locally available Mediterranean fish species. Food was offered two to three times a week at satiation levels. To establish a broodstock at the experimental site of the Institute of Marine Research in Iraklion about 10 fishes were transferred to Crete. Feeding was stopped 48 h before transporting the fish by truck. The transport lasted for about 35 hours. The transport tank was equipped with a technical oxygen advice, supplying pure oxygen via two airstones. The oxygen supply had to be regulated manually and water oxygen concentration was kept at a saturation level of 100-130%. During a first shipping period of about 10 hours (Leros-Rhodos) no water exchange was conducted. Thereafter, a continuous water exchange took place during an 8-10 hours stay on the island of Rhodos. During the last ship passage (Rhodos-Crete) only oxygen was supplied to the tank. The exchange of water on ferry boats is not to recommend, since the supply pipes are often spoiled by rests of oils, thus endangering fish health.

The dentex reared in Crete during 1993 and 1994, which had attained an age of almost two years and a body weight of 500-1000 g were transferred in spring 1995 to a bream-bass hatchery in the north of Greece to establish a first broodstock of hatchery raised fish. A total of 31 fish were transported in two square shaped polyester tanks installed on a truck. Each tank had a water volume of 3000 L and was supplied with two airstones at the bottom of the tank, during which the tank water was enriched with pure oxygen. After the tanks had been disinfected with Sodium - carbonate and afterwards thoroughly cleaned, the fish were netted from their raising tanks one by one and introduced into the transport tank from the door on the topside of the tank. The final stocking density in the tanks varied between 4-5 kg/m<sup>3</sup>. Initially, the tankwater was highly supersaturated with oxygen at levels of 200-280%. Afterwards no more oxygen was added for the next 8 hours until the oxygen saturation had declined to values of 120%-160%.

No water was exchanged during the 15-hours lasting transport. On arrival at the fishfarm, the water of the transport tanks was slowly exchanged with fresh filtered seawater during a period of about 2 hours. This slow exchange was necessary to acclimate the dentex to the lower ambient temperature at this site (16.5  $\Rightarrow$  13.5 °C). Afterwards, the water level was reduced and a narcotic at a weak dosage (100-150 ppm Ethylen-methyl-amin) added. In this way the fish

could be netted and transferred in water filled buckets into the prepared broodstock tank (volume = 17 m<sup>3</sup>) without any damaging.

### 3.6 Tank types and dimensions

The different tank systems employed in this study were chosen to support the requirements of different life stages and meet the requirements of the varying experimental designs. Since tank lay-out and design were of minor importance with regard to the scientific aims of this study, no detailed description of the individual tanks systems is given here. However, tank dimensions and construction material as well as the rearing phase during which they were

**Table 4:** Types and dimensions of tanks employed during the rearing and experiments with common dentex - larvae, -juveniles, - ongrowing and broodstock - fish. Tank diameter, water height, Volume (Vol.), the tank bottom slope and the tank material (P = Polyester; C concrete) are given. EP = experimental period.

	Diameter (cm)	Water height (cm)	Vol. (l)	Bottom slope (%)	Material	EP
Egg incubator	100	50	492	130	P	4; 7
Mesocosmos	500	200	40000	2-3	P	4; 7
Circular flat	110	50	450	2-3	P	1;2,5;8
Circular high	110	140	2000	1-2	P	6; 9
Cylindro- conical	110	100	2000	130	P	7
Broodstock 1 (square)	300	90	8100	2	C	3
Broodstock 2 (circular)	300	140	9900	3	P	9

employed are summarized in Table 4. The circular tank types as well as the square broodstock tank were equipped with a single point source water inlet and a central drain pipe. Through this arrangement, a circular water current was produced, supporting the self-cleaning mode of operation. It is important to note that the current had to be carefully adjusted to the swimming ability of different sized fish.

### 3.7 Water quality control

Unfavourable, as well as strongly fluctuating water quality conditions can considerably influence growth performance, fish behaviour and disease susceptibility in cultured fish (Fivelstad et al., 1993, Handy and Poxton, 1993, Wickins, 1981, Kawamoto, 1961). In order to evaluate the influence of such effects on the overall performance of the fish, several basic water quality parameters were monitored in regular intervals during the conducted experiments. Due to the relative low stocking densities in the test tanks and the high flushing

rates, which were maintained during most of the rearing trials and due to the applied flow-through system, accumulation of nitrogen metabolites to physiologically critical values was not anticipated. However, such factors were also determined.

### **3.7.1 Dissolved Oxygen**

Oxygen content of the rearing water was monitored once daily at 15:00 during all experiments and all larval rearing periods. The determinations were carried out by using a portable dissolved oxygen-meter (Yellow Springs Instruments Co., Model 57). During the mesocosmos larval rearing an additional oxygen reading was conducted in the early morning to observe possible oxygen deficiencies that may have occurred over night due to algae blooms. During broodstock transfer, oxygen was monitored frequently (20 min. - 2 h intervals) employing a WTW 96 portable oxygen-meter. The oxygen meters were calibrated daily in a water saturated atmosphere as recommended by the producer.

### **3.7.2 Total Gas Pressure (TGP)**

The total gas pressure (TGP) is defined as the sum of the partial pressures of all gases in solution. When total gas pressure of an aqueous solution is equal to local barometric pressure (BP), equilibrium conditions occur. The gas saturation of a solution is commonly given as a percentage of local barometric pressure (BP):

$$\text{Total Gas Pressure (\%)} = \text{BP} + \text{TGP} / \text{BP} \times 100$$

When total gas pressure of a solution is greater than barometric pressure, the water is supersaturated and gases tend to come out of solution, often in form of microbubbles. Such conditions can cause gas bubble trauma (and eventually gas bubble disease) in organisms living in such supersaturated water. Weitkamp and Katz (1980) have given an extended review on the subject and outline consequences of gas bubble disease in fish and fish culture.

In this study, Total Gas Pressure (TGP) was measured with a Weiss Saturometer (ECO-Enterprises, Seattle, Washington). This instrument consists of a long, small diameter, gas permeable, dimethyl - silicone tubing which is connected to a low - volume pressure gauge. When introduced into the water, dissolved gases diffuse through the membrane until an equilibrium between the dissolved gases in the water and the atmospheric gases is achieved. The pressure difference across the tube membrane is read on the pressure gauge, relative to atmospheric pressure, in millimeters of mercury. Total gas pressure was measured during an accidental occurrence of gas bubble trauma in broodstock fish (EP 9).

### **3.7.3 pH**

The pH values were relative constant in the sea-water flow-through system and, therefore, routine measurements were conducted in weekly intervals only. During larval rearing periods in still water (so called Mesocosm), which were commonly characterized by dense plankton blooms and low water exchange rates, pH values were recorded daily. A combined pH and temperature sensor (C 925, Consort) was employed, which was calibrated with WTW standard pH 4 and 9 buffer solutions in weekly intervals.

### 3.7.4 Salinity

Salinity was determined by a hand-refractometer from Atago. During the experiments 1, 2 and 5 daily measurements were conducted. During the other periods weekly measurements were conducted. Accuracy of determination:  $\pm 1$  ppt.

### 3.7.5 Temperature

Water temperature was determined daily in the morning (8:00 - 9:00). Daily fluctuations during experimental periods (summer) were found to be low and ranged between 1 - 2 °C in flow through rearing systems, applying high water exchange rates (30%-180%/hour). Temperature was determined with an accuracy of  $\pm 0.1$  °C.

### 3.6.6 Total ammonia ( $\text{NH}_4^+ + \text{NH}_3$ )

Total ammonia concentrations were determined during experiment 6 in weekly intervals, and in irregular intervals during experiment 8. The chemical analysis was conducted using the "Hypochlorite-Phenol-Method" after Koroleff (1970). Phenol, hypochlorite, and un-ionized ammonia react under strongly alkaline conditions to form indophenol, an intensely blue compound. The reaction is catalyzed by sodium nitroprusside. The intensity of the blue colour is measured spectrophotometrically (wavelength = 690 nm) and reflects the concentration of total ammonia in the sample.

Water samples of 8 ml were mixed with the reagents (1.2 ml Buffer solution, 0.3 ml Phenol reagents, 0.3 ml Hypochlorite solution), and afterwards stored for 24 hours in darkness at room temperature 20 - 25 °C to allow the colour complex to full develop (modification after Meyer, 1995). A standard curve with a stock ammonia-nitrogen solution was used to calculate concentrations from spectrophotometric readings. Duplicate determinations were carried out separately for every tank. Water samples were collected always in the afternoon (15:00 - 17:00) from the drainage water of the tanks.

### 3.7.7 Nitrite ( $\text{NO}_2$ )

Nitrite concentrations were determined after the "Sulfanilamide-Naphthylamin-Method" (German standard methods). Under acid conditions, nitrite reacts with sulfanilamide, a diazotizing reagent, to form a diazonium salt. The diazonium salt is coupled with the Naphthylamin reagent, an aromatic compound, to form a coloured pinkish red azo dye. The intensity of the pinkish red colour is measured spectrophotometrically (wavelength = 542 nm) and reflects the concentration of nitrite ( $\text{NO}_2$ ) in the sample. Water samples of 10 ml were mixed with the reagents, and afterwards stored for 30 minutes in darkness at room temperature 20 - 25 °C until the colour complex had been fully developed (Meyer, 1995). A standard curve with a stock nitrite-nitrogen solution was used to calculate concentrations from spectrophotometric readings. The sampling procedure was identical to the ammonia measurements.

### 3.7.8 Total suspended solids (TSS)

Suspended solids were determined after a standard method using pre-weighed membrane-filters of 0.45  $\mu\text{m}$  pore size (Sartorius) to filter a defined amount of sample water (200 ml). The weight difference of these filters before and after filtration is given as the amount of total suspended solids in mg/l. Filters were dried at 55 °C until constant weight.

### 3.8 Water flow measurements

Water flow rates have been measured by determining the time needed to fill a vessel of defined volume with either the inlet or the drain water of a fish tank. Respective flow rates are given in m<sup>3</sup>/hour.

### 3.9 Calculation of growth and food conversion factors

The exact estimation of growth and food conversion rates was among the main objectives of the study in order to evaluate the efficiency of different rearing and feeding regimes during most of the conducted experiments. Therefore, several factors were calculated from the data obtained on weight gain and food consumption to describe growth performance. However, the need for frequent growth measurements contradicts the susceptibility of fish to repeated handling stress. The high susceptibility to handling stress in common dentex was already indicated during the first screening period in 1992. In all experiments conducted later, the possible influence of handling stress on growth performance and disease susceptibility was thoroughly considered and type and frequency of measurements were carefully adapted to the main objectives of the respective investigation. For the estimation of food conversion rates, the feed consumption was recorded continuously.

**Table 5:** Growth and food conversion factors. SGR = Specific Growth Rate; FC = Food Conversion factor, ECR = Energy Conversion Rate; FR = Food Ration; CV = Coefficient of Variation; CF = Correction Factor for food loss.

$$SGR (\%/bw) = \frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{time (days)}} \times 100$$

$$FC = \frac{\text{feed dry weight (g)} \times CF}{\text{biomass wet weight increase (g)}}$$

$$ECR = \frac{\text{feed energy (KJ)} \times CF}{\text{biomass wet weight increase (g)}}$$

$$FR (\%/bw) = \frac{(\text{feed dry weight (g)/day}) \times CF}{\text{instant biomass}} \times 100$$

$$CV (\%) = \frac{SD (\text{standard deviation})}{x (\text{arithmetic mean})} \times 100$$

CF dry pellet = 0.25 (range: 0.203-0.296); CF moist pellet = 0

The food loss, in this study defined as the difference between the amount of food offered to the fish (crude food consumption) and the amount that is consumed by the fish (net food consumption), was a rather difficult parameter to measure. Food loss can vary considerably

depending on type of food used, feeding strategy, feeding frequency, fish size and age. Changes in appetite can also influence the results. Thus, food loss had been estimated and a correction factor be determined in order to be able to calculate of net food consumption in moist and dry diets (3.8.1  $\Rightarrow$  Food loss). These factors, although only rough estimates, were used to correct the recorded food consumption rates, leading to more realistic calculation of the true consumption rates and conversion factors (Table 5).

Growth was described by - individual wet weight increase, - biomass increase, - total length increase, and the - specific growth rate (SGR %). The food conversion was expressed as food conversion - (FC) and - energy conversion rate (ECR). Food consumption was estimated as amount dry food fed per day as percentage of fish biomass (food ration). The size variability of tank populations was described by the coefficient of variation (CV %). The calculation of the growth and food conversion factors is described in Table 5.

The instant fish biomass was determined by interpolations from growth data of two subsequent weightings and correcting for daily mortalities, considering the mean weight of the previous weighing as the one for the dead fish.

Daily feeding activity is shown as the part of food consumed at one of the daily feeding periods as percentage of the total daily food ration.

### ***Food Loss***

Food loss was determined during this study in three ways: (a) collecting the uneaten feed pellets and particles from the bottom of the tanks with a siphon equipped with a fine mesh net (100  $\mu$ m opening), (b) collecting and back-weighing the remaining feed from the automatic conveyer belt feeder and (c) determining the difference of total suspended solids (TSS) between tank inlet and tank outlet water 30 minutes after feeding was terminated.

The food matter collected was dried at a temperature of 55 °C until constant weight and weighed. To reduce the contamination of feed samples with faeces during the measurements of (a) and (c), the respective tanks were cleaned by siphoning shortly before feeding started and the sampling procedure took place shortly after feeding had finished (about 30 minutes).

The estimation of food loss was conducted for the dry pellet only during the experimental period (EP) 5, as described under (a) and (b). During this experiment the food loss of the moist diet was assumed to be negligible due to the applied feeding regime (hand-feeding to appetite). During EP 6 it was planned to estimate the food loss of different moist diets by method (a) and (c). However, the high variability of the suspended solid load between samples from the water column (inhomogen distribution pattern), and the time fate of particle production during and after feeding requires a relative high number of measurements in order to estimate reliable food loss levels. Since the number of determinations of suspended solids was limited during this study, no reliable data on food loss of different moist pellets were obtained. Food consumption and conversion factors on moist diets base, therefore, in all experiments on the amount of food administered to the fish without any correction for food loss (Table 5).

### 3.10 Food composition and processing

As no reliable information on feed preferences and nutritional requirements of the common dentex was available, a variety of fresh - and moist - diets were prepared on site.

Fresh diets were fed to juveniles only during the very first rearing period (EP 1). These diets were prepared from different fish species (Scombrids, Clupeids, Sparids), squids (*Sepia officinalis*, *Octopus vulgaris*) and shrimps (*Penaeus spec.*), which were bought from the local fish market and cut into pieces suitable for the direct uptake by the fish. Broodstock fish were fed mainly with frozen fish and squids. Once, live gilthead seabream (20-30 g) were fed to broodstock fish in order to stimulate the predatory behaviour and thus initiate feeding, and this was done during a period of appetite loss (after transfer  $\Rightarrow$  3.5).

Fresh to moist diets were subsequently offered during the weaning of early juveniles from live food organisms (*Artemia*, Copepods) to a moist or dry pellet. Weaning started with fine minced fish filet, followed by a paste composed of 40 % minced fish filet + 30% minced shrimp meat + 30% starter food (LANSY, by Artemia Systems, Gent; Table 6). When most of the juveniles started feeding on this paste, the part of shrimp meat was subsequently replaced by the cheaper fish meat.

Moist pellets were prepared from deep-frozen fish products and commercially available seabream/ sea-bass pelleted diets (Table 6). Initially these moist diets contained about 30 % of dry pellet and 70 % of fish filet (EP 1), subsequently increasing the pellet part up to 70 % and decreasing the fish content down to 30 % (EP 2). During 1993 (EP 5) the moist pellet had a pellet (P) and fish (F) share of 25% (P): 75% (F) and in 1994 a mixture of 55% (P): 45% (F) (EP 6,8,9) was fed. The relationship between pellet and fish compounds of the 1994 moist pellet was found to give an optimal stability during processing and feeding.

As nothing was known about the specific requirements of this species for vitamin, mineral or essential fatty acids, moist diets were supplemented with commercial vitamin mixtures (Vitaflush, Aquace) and a HUFA fish oil (Aquatac) at concentrations recommended by the producer for other carnivorous species. During the first year, 2 g Vitamin C (ascorbic acid) per kg food was added. This was meant to function as an anti-stress agent during the experimental periods 1 and 2. The characteristics of the applied commercial and mixed moist diets are summarized in Table 6.

Dry pellet were stored as recommended by the producer at a dry and shadow place. The fish and pellet components of the moist pellets were mixed, once grinded and enriched with a vitamin mix and a HUFA oil. This feed mixture was stored at -20 °C in small batches. Shortly before feeding, these feed batches were taugth and portions, sufficient for 2-3 days, pelleted via a grinder. In experiments 8 and 9 pulverized  $\beta$ -glucans and in experiment 6 tape water was added at different concentrations directly before feeding.



	Food fish		Dry pellets			Moist diets	
	Boobs boobs	Trachurus mediteraneus	Ecostart 17	Lansy 4	Aqualim 2	Moist pellet 93	Moist pellet 94
Size range (mm)	variable	variable	1.5	0.8 - 2	2	3 - 10	3 - 10
Composition: Pellet : fish	0 : 100	0 : 100	100 : 0	100 : 0	100 : 0	25 : 75	55 : 45
Moisture (%)	73	76	7	7 - 10	6 - 8	50-52	35-40
Energy content (MJ/kg dry matter)	22.64	23.92	21.9	23.76	21.21	23.35	22.53
Protein (%)	86.38	71.57	47	48	52.57	-	51.35
Lipids (%)	5.05	16.62	20	14.5	12.67	-	18.39
Ash (%)	5.87	9.30	8.57	10.2 - 11.0	10.83	12.53	11.18
Commercial additives							
Aquatac <sup>1</sup> (ml/kg)			-	-	-	15	15
Aquace <sup>2</sup> (g/kg)			-	-	-	2	2
Vitamin C (g/kg)						2	
Gelatin Binder (%)			-	-		-	0.5

<sup>1</sup> Aquatac contains a minimum of 20% polyunsaturated fatty acids (specially 10:5  $\omega$ 3, 22:5  $\omega$ 3, 22:6  $\omega$ 3) and quantities of 20:4  $\omega$ 6 fatty acid; <sup>2</sup> Aquace contains per kg: 2.5 Mio. I.U. Vitamin A, 250 g Vitamin C (Ascorbic acid), 125 g Vitamin E and Vitamin B1, B2, B6, B12 in unknown amounts.

### 3.11 Feeding regime

*Preliminary rearing trials (EP 1 & 2) showed that sub-optimal feeding frequencies in juveniles caused a considerable increase in mortality due to agonistic behaviour. Jobling (1983) states that nutritional trials on restricted feeding rations do not allow a reliable comparison of different feed qualities.* Therefore, in this study, feed ration was regulated mainly by appetite (ad libitum feeding) and feeding frequency was chosen by defining minimum intervals between two feedings, which would not lead to increasing agonistic behaviour in early juveniles (<10 g). In larger fish (> 10-20 g), the interval between two feedings was prolonged, when the amount of food consumed during two subsequent feedings was markedly reduced. Due to the decreasing metabolic rates of larger fish, the feeding frequency was reduced with growth as shown in Table 7. Dry pellets were fed on automated conveyer-belt feeders (1,2,5), whereas fresh diets and moist pellets were fed by hand (4,6,7,8,9). Feeding started 1-2 hours after sunrise (7.30) and continued for 10-14 hours. Broodstock fish were fed daily to every 3. day, respective to ambient temperature and reproduction period.

Hand feeding allowed to estimate very precisely the food consumption and conversion rates, because in contrast to automated feeder systems, food loss due to variable appetite is very low.

**Table 7:** Approximate relationship between feeding frequency (number of meals per day) and fish size in juvenile and ongrowing common dentex fed with high energetic - (> 21 MJ/kg), high-proteinous- (>47% protein) diets at ambient temperatures of 23 °C to 25 °C. Fish were fed by hand, in regular intervals during day time until satiation.

Fish weight (g)	0.1-0.5	0.5-2g	2-10	10-40	40-100	100-500
Fish length (mm)	20-33	33-52	52-87	87-136	136-183	183-308
Feeding frequency (meals/day)	8	6	5	4	3	2

During the experiment on the effects of immunostimulation (EP 8), the first week, and subsequently every second week the moist pellets were enriched with  $\beta$ -glucans according to the respective test groups. Control fish were fed non-treated reference diet only.

### 3.11 Pellet analysis

#### 3.12.1 Moisture content

The moisture content of the different feeds was determined by drying a 1-2 g sample at 105 °C to constant weight. The difference in weight before and after drying was defined as the moisture content in percent. Data are given as mean values of triplicate measurements.

#### 3.12.2 Ash content

Feed ash content was determined by burning a 1-2 g sample at 500 °C till constant weight. The remaining non-organic matter was defined as the ash content and expressed as percentage of the pellet dry matter.

### 3.12.3 Crude protein

The analysis of crude protein was carried out by the "Institut für Tierernährung und Stoffwechselphysiologie" after the German official method for chemical analysis of feed compounds (Bassler and Buchholz, 1993). This method is used to determine the crude protein content in feed compounds, calculated from the nitrogen content analyzed after Kjeldahl. The determination of the total protein content is based on the assumption that proteins do contain a mean of 16% nitrogen. The protein content is then expressed by the multiplication of the determined nitrogen content with a factor 6.25.

#### Principle of the method.

The sample is broken down with sulfuric acid in the presence of a catalyst. The resulting acidic solution is alkalized with sodium leach. Afterwards, the released total ammonia is distilled into a retort, filled with a defined amount of sulfuric acid, of which the surplus is titrated with a sodium leach of a defined concentration. The content of crude protein in percentage of the sample is calculated after the following equation:

$$\text{Crude Protein (\%)} = \frac{(V_0 - V_1) * c * 0.014 * 100 * 6.25}{m}$$

$V_0$  = Volume (ml) of sodium leach used for the titration of the blind sample

$c$  = Concentration (mol/l) of the sodium leach

$V_1$  = Volume (ml) of the sodium leach used for the titration of the sample

$m$  = mass (g) of sample

Samples containing more than 40 % crude protein should not deviate more than 0.4% in two parallel determinations.

### 3.12.4 Crude lipids

The analysis of crude lipids was carried out by the "Institut für Tierernährung und Stoffwechselphysiologie" after the German official method for chemical analysis of feed compounds (Bassler and Buchholz, 1993)

The sample is treated with hot hydrochloric acid, afterwards this mixture is cooled down and filtered. The washed and dried residue is extracted with petrol ether. The solvent is distilled off and the residue dried and weighed. The amount of residue will be given as percentage of the sample. Samples containing more than 10% crude lipids should not deviate more than 0.4% in two parallel determinations.

### 3.12.5 Pellet energy content

The energy content of pellets was determined by an adiabatic bomb-calorimeter (Phillipson oxygen micro bomb calorimeter, 2% accuracy). In this method the heat, which is released when a defined amount of organic matter is completely burned in an pure oxygen atmosphere (20 bar pressure), is determined. The quantitative burning of a previous dried and weighed amount of organic matter is carried out in a thick-walled steel vessel, which contains a small defined amount of distilled water. The heat increment of this water is directly correlated with

the energy content of the probe and is measured by an electrode which is connected to a recorder. Calibration of the bomb calorimeter and the recorder was done by burning exact amounts of a substance of a known energy content. Energy content in MJ/kg dry weight can be calculated taking several correction values and the ash content into account. For each different test pellet 5 parallel determinations were taken.

### 3.13 Weight and length measurements

Growth was commonly measured as wet weight gain. Individual specimens (EP 2,3,5), sub-samples of the tank population (EP 5) or whole tank populations (EP 1,6,8) were weighed. Before sampling fish, the water level in the tank was reduced to 20-30 cm, to avoid a selective netting due to differences in escape capacity between different sized fish. For individual measurements, fish were anaesthetized with Ethylene-glycol-monophenyl-ether ( $C_8H_{10}O_2$ ) at a concentration of 0.15-0.4 ppm, then placed on filter paper to remove external water, and thereafter immersed in a water filled, tarred vessel on an analytical balance (Sartorius  $\pm 0.001$  g). Sub-samples and whole tank populations were weighted in a similar way, but no filter paper was employed for drying and fish were not anaesthetized. Weighting of whole tank populations during experimental period 8 caused very high densities of more than  $100 \text{ kg/m}^3$ , which were applied to generate repeatedly an intensive stress level. During EP 6, a larger digital balance ( $\pm 100$  g) was employed because total weights of water filled bucket + fish exceeded the limits of the available analytical balances ( $>40$  kg).

### 3.14 Morphometric measurements

Morphometric data analysis was conducted to a) establish regressional relationships between individual length-weight characters over a wide size range, and b) identify allometric growth patterns of life stages of this species investigated. The general suitability of morphometrical data analysis for the above summarized objectives have previously been shown to work in studies of several authors using other fish species (Fukuhara, 1992; Osse & van den Boogaart, 1992; Theilacker, 1987; Fuiman, 1983).

Eleven length and weight characters were recorded from random fish samples. These samples were collected with the weekly weight measurements during Experiment 5. The data-set includes weekly measurements on 90 fish (15 fish from 6 tanks) over a 7 weeks period. Additionally, measurements from dead fish, collected during the experimental periods 4,6 and 7 (for experimental periods see Table 2 and Figure 2) were also conducted, to draw a more complete picture of morphometrical development, including a wider size range of fish and to determine whether moribund fish show immediate differences to healthy and ongrowing fish. Due to the wide size range of fish investigated, different tools for the length and weight measurements were employed. The type and accuracy of these tools are summarized in the following paragraph.

Weight measurements were conducted on an analytical balance (Sartorius) ( $\pm 0.00001$ g) for postlarvae and early juveniles (0.01g - 1.00g) as well as for the determination of liver-, spleen-

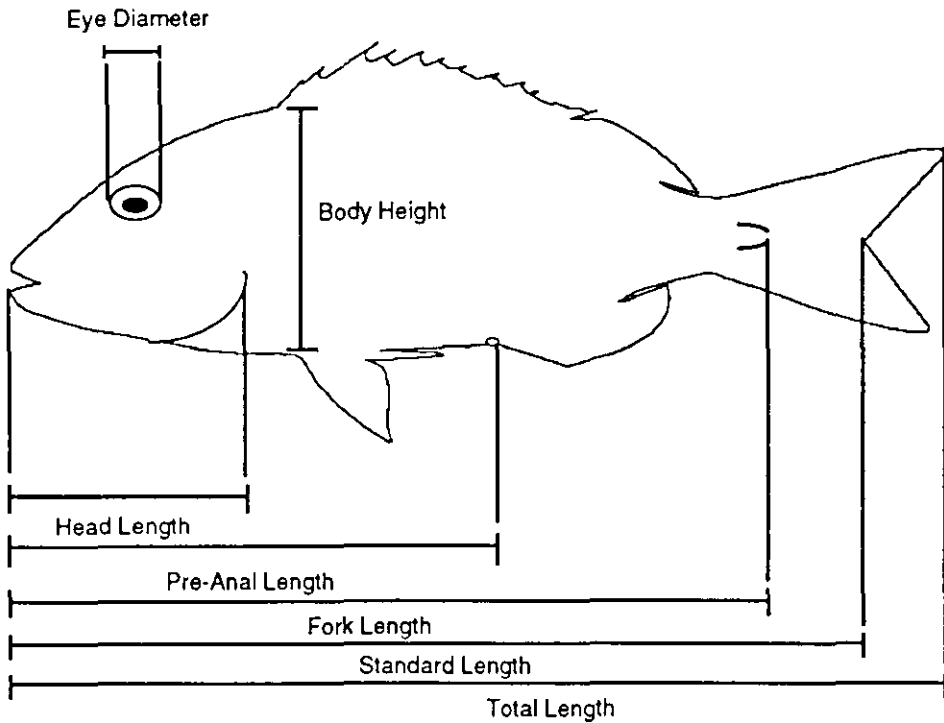
depended on fish age, temperature and stage of maturation (Heidinger and Crawford, 1977; Haider, 1984; Bolger & Connolly, 1989; Adams and McLean, 1985). The gonado-somatic index is widely used to describe the stage of maturation in fish (Adams & McLean, 1985; Donaldson, 1990). The mesenteric fat index (MFI) is a rather uncommon index, probably due to the relative labour intensive sampling of fats between the intestine and organs, which can cause a higher inaccuracy compared to other somatic indices. However, since fat depots are indicating a surplus of the feed energy - metabolism balance, the relative amounts of stored fat could give some information on the suitability of the administered food. An index including these fat depots, is the "Gut index" introduced by Jensen (1980), who determines the relative energy content, or the dry weight of the rest of the intestines, after removal of the gonads, swim bladder and liver in brown trout (*Salmo trutta*) and Arctic char (*Salvelinus fontinalis*).

**Table 8:** Equations for the calculation of organo-somatic factors and indices employed during the data analysis of the present study.

$$\begin{aligned}
 \text{Condition Factor (CF)} &= \frac{\text{Total weight (g)}}{\text{Total length (cm)}^3} \times 100 \\
 \text{Liver Somatic Index (LSI)} &= \frac{\text{Liver weight (g)}}{\text{Karkass weight (g)}} \times 100 \\
 \text{Spleen Somatic Index (SSI)} &= \frac{\text{Spleen weight (g)}}{\text{Karkass weight (g)}} \times 100 \\
 \text{Gonado Somatic Index (GSI)} &= \frac{\text{Gonad weight (g)}}{\text{Karkass weight (g)}} \times 100 \\
 \text{Mesenteric Fat Index (MFI)} &= \frac{\text{Mesenteric fat (g)}}{\text{Karkass weight (g)}} \times 100 \\
 \text{Intestinal Length Index (ILI)} &= \frac{\text{Intestine length (cm)}}{\text{Total length (cm)}}
 \end{aligned}$$

The intestinal length index (ILI) is a mean to describe the relative length of the intestine in relation to total length and can serve information on natural feeding behaviour during different life stages. (Kapoor et al., 1975; Weatherly & Gill, 1987).

The above described measurements were determined from 60 juveniles, which were sacrificed at the end of experiment No. 5. (10 fish per tank). Additionally, the same measurements were taken from 20 fish, which were sampled at the end of experiment 6, and 9 accidentally dying broodstock fish. From the later, also the gonads were weighed and the gonado-somatic index determined. Spleen weights were taken in 10 individuals during EP. 6. The organo-somatic factors and indices were calculated as described in Table 8.



**Figure 4.** Schematic drawing of a juvenile common dentex. Seven morphometric length measurements, taken from juvenile and adult common dentex are indicated by the named bars.

and mesenteric fat- weight. Larger juveniles up to an individual weight of 150 g and gonads were weighed on a balance ( $\pm 0.01$ g). Groups of ongrowing fish (EP 6) were weighed on a digital balance of  $\pm 100$  g accuracy. Length measurements of larvae ( $< 10$  mm TL) were carried out with an ocular micrometer ( $\pm 0.0001$  cm), using a microscope (4-10 x magnification); postlarvae and early juveniles (10-25 mm TL) were measured employing a binocular (2-4 x magnification) and graph paper ( $\pm 1$  mm), larger fish were measured by ruler ( $\pm 1$  mm) or measure wood ( $\pm 10$  mm). Mouth opening and eye diameter in juveniles and ongrowing fish were measured using a capilar ruler ( $\pm 0.1$  mm). The mouth opening was determined as maximum mouth opening. For measurements, a capilar ruler was introduced into the mouth gap and the vertical distance between the tip of upper and lower jaw determined. Especially in smaller individuals, this procedure requires some care, not to force the mouth opening to levels exceeding the physiological range. The functional mouth opening is considered to be considerably smaller than the maximum mouth opening (Hunter and Krimbel, 1980).

### 3.15 Organo-somatic-indices

Several organo-somatic indices were calculated to describe (a) their size-range in cultured common dentex, (b) identify possible changes at different stages of the life cycle. The meaningfulness of factors and indices employed during this study are briefly summarized in the following paragraph.

The condition factor after Fulton is a commonly used term, indicating a mid-long term nutritional stage (Goede and Barton, 1990; Adams & McLean, 1985). The Liver-Somatic-Index (LSI) responds more rapidly to environmental and metabolic changes, but is highly

depended on fish age, temperature and stage of maturation (Heidinger and Crawford, 1977; Haider, 1984; Bolger & Connolly, 1989; Adams and McLean, 1985). The gonado-somatic index is widely used to describe the stage of maturation in fish (Adams & McLean, 1985; Donaldson, 1990). The mesenteric fat index (MFI) is a rather uncommon index, probably due to the relative labour intensive sampling of fats between the intestine and organs, which can cause a higher inaccuracy compared to other somatic indices. However, since fat depots are indicating a surplus of the feed energy - metabolism balance, the relative amounts of stored fat could give some information on the suitability of the administered food. An index including these fat depots, is the "Gut index" introduced by Jensen (1980), who determines the relative energy content, or the dry weight of the rest of the intestines, after removal of the gonads, swim bladder and liver in brown trout (*Salmo trutta*) and Arctic char (*Salvelinus fontinalis*).

**Table 8:** Equations for the calculation of organo-somatic factors and indices employed during the data analysis of the present study.

$$\begin{aligned} \text{Condition Factor (CF)} &= \frac{\text{Total weight (g)}}{\text{Total length (cm)}^3} \times 100 \\ \text{Liver Somatic Index (LSI)} &= \frac{\text{Liver weight (g)}}{\text{Karkass weight (g)}} \times 100 \\ \text{Spleen Somatic Index (SSI)} &= \frac{\text{Spleen weight (g)}}{\text{Karkass weight (g)}} \times 100 \\ \text{Gonado Somatic Index (GSI)} &= \frac{\text{Gonad weight (g)}}{\text{Karkass weight (g)}} \times 100 \\ \text{Mesenteric Fat Index (MFI)} &= \frac{\text{Mesenteric fat (g)}}{\text{Karkass weight (g)}} \times 100 \\ \text{Intestinal Length Index (ILI)} &= \frac{\text{Intestine length (cm)}}{\text{Total length (cm)}} \end{aligned}$$

The intestinal length index (ILI) is a mean to describe the relative length of the intestine in relation to total length and can serve information on natural feeding behaviour during different life stages. (Kapoor et al., 1975; Weatherly & Gill, 1987).

The above described measurements were determined from 60 juveniles, which were sacrificed at the end of experiment No. 5. (10 fish per tank). Additionally, the same measurements were taken from 20 fish, which were sampled at the end of experiment 6, and 9 accidentally dying broodstock fish. From the later, also the gonads were weighed and the gonado-somatic index determined. Spleen weights were taken in 10 individuals during EP. 6. The organo-somatic factors and indices were calculated as described in Table 8.

### 3.16 Disease recognition and treatment

Several times during the rearing of juvenile and ongrowing dentex, epidemic infections, with subsequent high mortalities were observed. Routinely, gill and skin smears were prepared and microscopically examined from live infected fish. Internal organs were dissected and investigated by binocular observation; liver and spleen tissue were microscopied and the body cavity as well as the intestine content examined for macro-parasites or aberrations.

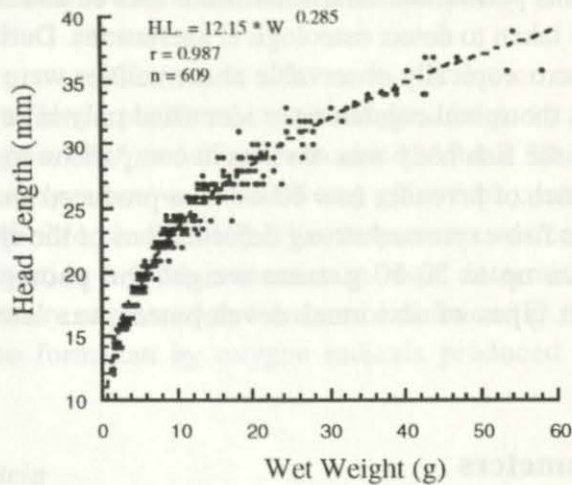
In the case of bacterial and micro-protozoal caused disease, antibiotics were applied as bath or orally via the feed. Antibiotic baths had to be employed when fish were already hardly infected and accepted no feed. A nitrofurantoin (Furaltadon, Merck 11, 4205), combining antibacterial and anti-protozoal activity, had been employed at concentrations between 50 - 150 ppm. To limit the volume of administered antibiotics, water level were reduced down to 15-20 cm during the treatment, which commonly lasted 60 - 120 minutes. Water exchange was stopped and each tank was supplied with pure oxygen during this period. If fish showed signs of irritations, the antibiotic concentration was reduced rapidly by water exchange. Oxytetracycline HCl (a tetracycline) was applied orally at a concentration of 80 mg/kg body weight /day. The antibiotic was applied via a moist pellet, which revealed good mixing properties, providing uniform distribution of the therapeutants within the feed portions. The visual observation of fish during the manual feeding allowed also the control of appetite in individual diseased fish.

Parasites were treated by formalin (100 - 250 ppm) and copper sulfate (0.5 - 1.5 ppm) baths. In this way, protozoan parasites could be effectively controlled. A standardized treatment procedure was set up during a micro-protozoal infection in experiment 8. After the first fish in a tank died from infection, fish were bathed in formalin (200 ppm) for 30 minutes and subsequently in a copper sulfate ( $\text{CuSO}_4$ ) solution (1 ppm) for 15 minutes. The following two days only copper-sulfate treatments were employed, while at day 4 a final combined formalin-copper-sulphate treatment took place. This treatment schedule was continued as long as mortalities due to the disease occurred.

### 3.17 Determination of mortality

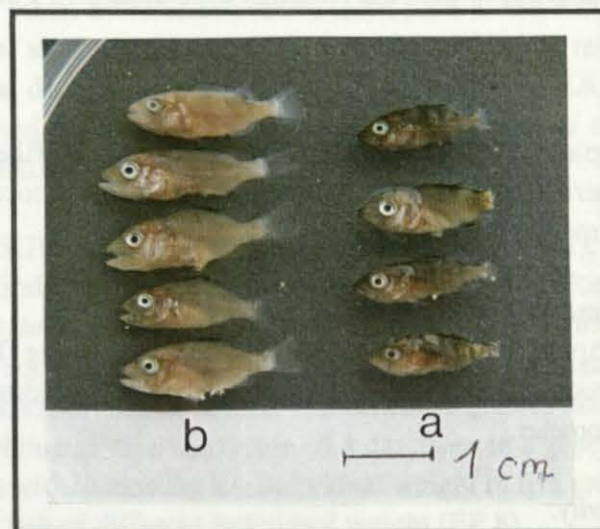
Mortalities were recorded every morning and during feeding in all experiments with juvenile and ongrowing fish. Dead fish were removed from the tanks and examined for mechanical damages and obvious infections on body surfaces and gills. During all experimental periods, dead fish expressing injuries, typically caused by biting attacks of tank-mates, were recorded separately as mortalities related to agonistic behaviour. Agonistic behaviour has been defined for the purpose of this study as repeated attack (particularly biting) by conspecifics. This agonistic behaviour can be classified as a filial (when coming from parents) or sibling- (different parents) intracohort relationship, similar to the classification of cannibalism by Smith and Reay (1991). Due to the frequently missing tail in dead fish (biting attack  $\Rightarrow$  Figure 6), total or standard length could not be used to assess the size class of these fish and the head length was determined instead (EP 5). Previous measurements established a strong correlation between head length and body wet weight with this relationship being used to back-calculate the size of moribund and dead fish (Figure 5).





**Figure 5.** Relationship between wet weight and head length in juvenile *Dentex dentex*. H.L.- Head Length, W - wet weight, r - correlation coefficient, n - number of fishes.

Ulcerative occurrences, eye damages and other kind of pathological abnormalities were recorded. During experiment 8, mortis were separated into 4 different categories: (a) no external visible damage, (b) eye damage, (c) tail damage, (e) gills infected. The tail damage was related to agonistic biting behaviour (Figure 6); described eye damage include all macroscopically observable anomalies such as exophthalmia, clouding of the cornea and loss of one or both eyes. Gill damages were easy to recognize and were probably due to a protozoan infection. During EP 5 dead fish were separated into two categories only: (1) died by agonistic behaviour and (2) died due to other unknown causes (Figure 6). Mortality rates were expressed as percentage of the initial number of fish stocked during a defined time period.



**Figure 6.** Mortality in juvenile common dentex related to (a) agonistic biting behaviour and (b) natural mortality. The missing tail fin and the darker colouration of the attacked fish was typical and easy to identify. Note, the attacked fish are smaller when the fish which died due to unknown causes. Age: 49 days after hatching; Total length: 14-18 mm.

### 3.18 Monitoring of abnormal development

During the experimental period 1 and 2 in 1992, dead fish were collected and stored deep frozen. At the end of this period, the frozen fish were thawed and radiographs from a random sample of 45 fish were taken to detect osteological aberrations. During the production series in 1993 and 1994, all macroscopically observable abnormalities were recorded by photography. Thus, deformations on the spinal column were identified only in severe cases, when a clearly visible deformation of the fish body was obvious in comparison to normal developed fish. In spring 1993, a small batch of juveniles ( $n = 60-40$ ) was produced under intensive larval rearing conditions. All of these fish expressed strong deformations of the spinal column and the head. These fish were grown up to 30-50 g mean weight and photographed individually. The frequency of different types of abnormal development was later determined from these photographs.

### 3.19 Blood parameters

At end of the experiments 8 (testing the effects of different immunostimulants), blood samples were collected to measure a series of factors, which can serve as indicators of the non-specific immune system.

Blood samples were taken from 15 fish of each test tank at the end of the experiment to determine selected routine parameters and some response indicators of the non-specific defense system. Fish were collected by netting in groups of 5 and blood was taken from the caudal vein with a heparanized syringe. Only small blood volumes of 0.2-0.5 ml were gained per fish, largely depending on fish size. Blood volume obtained from one fish was not sufficient to perform all assays. Therefore, blood from three fish had to be used. To separate blood plasma from blood cells, samples were centrifuged at 6000 rpm in a Megafuge 1.0 R (Heraeus spatech). All spectrophotometric readings were done on a Pharmacia LKB-Ultrospec III.

#### 3.19.1 Haematocrit

From fresh blood samples two Haematocrit capillary tubes were filled and centrifuged in a Haematocrit micro centrifuge for 3 min. at 5000 rpm. The Haematocrit (%) was determined directly as percent volume of erythrocytes.

#### 3.19.2 Leukocyte numbers

From fresh blood samples a diluant (1:190) was prepared using Turks solution. (Turks solution destroys erythrocytes and slightly stains Leukocytes). Duplicate samples were counted in a Neubauer hemacytometer.

#### 3.19.3 Lysozyme activity:

0.1 ml of plasma were diluted 1:1 with PBS (phosphate buffered saline) and 0.5 ml of a suspension of *Micrococcus lysodeikticus* (Sigma, M3770) was added. The *Micrococcus* suspension was prepared to give a spectrophotometric transmission of 40% at 450 nm. Directly after mixing the plasma with the bacteria suspension, the kinetics of bacteria lysis

were followed by turbidity measurements in regular time intervals (1, 2, 3 and 30 min.). A standard curve was determined from egg white lysozyme (Sigma L6876). Results are expressed as units of transmission increase per minute and as percentage of transmission increase after 30 min. in relation to the transmission after 1 minute.

#### **3.1.4 NBT assay**

0.1 ml of blood were placed into a plastic micro centrifuge tube and 0.1 ml of an 0.2% NBT- (nitroblue tetrazolium; Sigma, 6876) solution were added. After a 30 min incubation period, 0.05 ml of the blood-cell suspension were added to a glass tube containing 1.0 ml of N,N-dimethyl formamide (DMF; Merck, 803068). This suspension was centrifuged for 5 min at 3000 rpm and the extinction of the supernatant was determined at 540 nm in a spectrophotometer. Results are expressed as measured extinction (E). High readings indicate that NBT was reduced to formazan by oxygen radicals produced from neutrophils and monocytes.

#### **3.19.5 Total serum protein**

Total serum protein was determined using a protein assay test kit (Sigma, P 5656) which based on the micro Lowry method. 10 µl of plasma were diluted (1:100) and reagents added, according to the test manual. Absorption of duplicate samples was measured at 750 nm. A calibration curve was prepared from bovine serum albumin, fraction V (Sigma, P 7656) and the respective protein content of the samples calculated.

### **3.20 Statistical analysis**

All data analysis were conducted using a statistical software package (SYSTAT, 1992). Data were tested for normal distribution using a Lillifors-test. This test standardizes the variable values and tests whether the standardized version is normally distributed. The Lillifors test has been chosen instead of other, more conservative tests, such as the Kolmogorov-Smirnov test for normal distribution, since Analysis of Variance (ANOVA) is relatively robust against deviations from normal distribution (Zar, 1984). One-way ANOVA (Model I, completely randomized experimental design) was used for the comparison of two or more means of normal distributed variables.

Specific growth rates (SGR) are strongly size dependent (Brett, 1971), thus SGRs determined during subsequent periods within an experiment were corrected for fish size, in order to represent the growth rate of a unit-size fish of 10 g. The size correction was conducted employing the linear equation:  $\log \text{SGR} = 1.131 - 0.438 \log \text{mean weight}$ , which was estimated during several experimental periods from specific growth rates of fish of a wide size range ( $\Rightarrow$  4.6). After Jobling (1983), the factor - 0.4 describes in a general way the decrease of log specific growth rate with increasing log individual weight in fish and can therefore be used to compare SGRs from fish of different individual weight (EP 8).

Growth curves were compared by regression analysis of logarithmic ( $\log_{10}$ ) transformed values of individual weight. Logarithmic transformation of fish weights plotted against time

resulted in significant linear regressions (EP 5). The regression slopes were compared using Student's t-test (Sokal & Rohlf, 1981). When regression lines of triplicates were not different, their pooled data were used in the final analysis. A two-sample t-test was employed to compare blood parameter during EP 8, after homogeneity of variances was tested by the variance ratio test (Zar, 1984). Comparing the Coefficient of variation (CV) and the relative size of "cannibalized" fish, the U-test (Mann and Whitney) for equal sample sizes was applied. Mortality rates, expressed as numbers of dead fish within different treatments (test-tanks), were compared by the  $\chi^2$ -test (Sokal and Rohlf, 1981) during all experiments. The skewness ( $g_1$ ), which was determined from fish size distributions (individual tank populations), obtained during EP 5 was tested for positive asymmetry ( $H_A: \gamma_1 > 0$ ) using critical values for skewness given by Zar (1984). A probability of 5% or below were used as criterion for the rejection of the null hypothesis in all tests.

## 4 RESULTS

### 4.1 First experiences during rearing trials with juveniles (EP 1 & 2a+b)

First experiences concerning behaviour under culture conditions and feed acceptance, when feeding a variety of different food qualities were gained during period one. Subsequently, two small-scale experiments of preliminary character, comparing a commercial dry pellet and a self prepared moist pellet, were conducted during period 2a and 2b. The aim of these studies were to largely standardize handling procedures and provide experimentals adapted for use in experimental design during subsequent periods of this investigation.

The juvenile dentex, which were first reared during the screening period (EP 1) and subsequently also used for first rearing trials (EP 2), were probably produced under unfavorable larval rearing conditions, and therefore expressed a high prevalence of specimens with severe skeletal deformations. The types of deformation, as well as their prevalence among the fish population of this rearing period are analysed in detail in chapter 4.8. The efficiency of important physiological processes such as swimming, breathing and feeding are possibly altered in fish expressing skeletal deformations of the head and the vertebral column. Also survival rates are often known to be reduced in such fish (Chatain, 1987; Barahona-Fernandes, 1982) and similar effects are likely to have occurred in our experiments. Therefore, the results on growth performance, normal mortality and disease susceptibility, gained during this experimental period should be considered as possibly deviating from the normal physiological response of unaffected specimens of the investigated species.

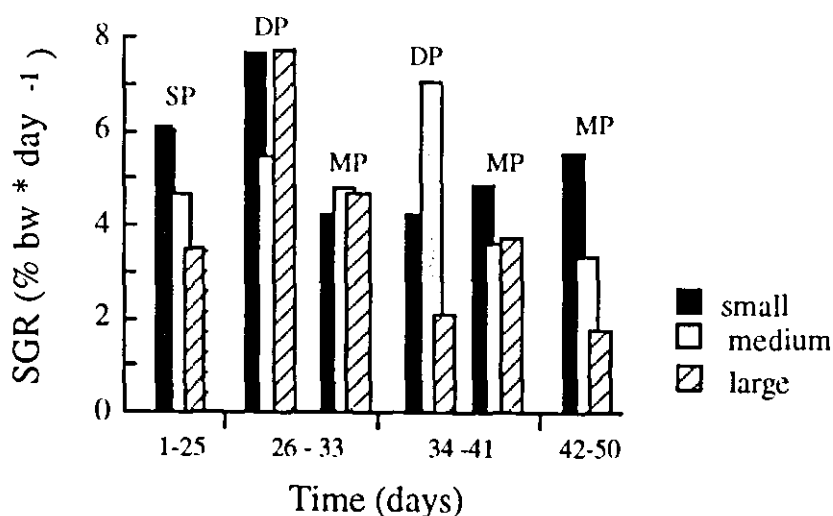
#### 4.1.1 Screening trial concerning food acceptance, feeding behaviour and routine handling procedures: Experimental Period (EP) 1

##### (a) Natural food

During the screening period a variety of fresh feeds of high quality were offered to the juvenile fish. Small pieces of fish filet, including a variety of locally available, low priced fish species (*Boops boops*, Sparidae; *Maena smaris*, Emmelichthyidae; *Trachurus mediterraneus*, Scombridae; *Sardina pilchardus*, Clupeidae), octopus flesh (*Octopus vulgaris*) as well as shrimp meat (*Penaeus kerathurus*) were readily accepted. Since no clear preference for any of these diets was observed, fish meat was finally chosen as a base for moist pellets, mainly for economical and practical reasons. A moist pellet, containing ingredients close to the composition of the natural diet of the common dentex was considered to be a reference diet during the subsequent rearing phases. Although shrimp meat has also a favorable composition and belongs to the natural diet of many Sparids, its relative high wholesale price makes its commercial use almost impossible. Thus, shrimp meat was used only for weaning purposes, when relative small quantities of high quality food were needed to feed juveniles. The use of *Octopus vulgaris* and other related cephalopods as practical diet under culture conditions, is also limited by their relative high wholesale prices in the Mediterranean area. Furthermore, the processing of the fibrous cephalopod meat was difficult. The production of homogen sized

mean weights and/or the fact that only moist pellet fish were handled during their transfer into the new tanks, and the dry pellet fish not. This could have caused reduced growth rates in the moist pellet fish due to handling stress.

During the second week of period 2a the specific growth rates of fish fed moist or dry diets reached similar levels, but the growth rate of the largest fish was considerably reduced, even below the level of the moist pellet fish of a similar size class. In the second half of this period, the medium sized fish of the dry pellet groups achieved the highest relative growth rates of 7.06 %. These fish became first infected after day 42. Due to the severity of the epidemic outbreak, which occurred only in the 3 tanks fed on dry pellets (Figure 10), no weights were taken during at day 50 (n.d. in Table 9). Feeding continued with moist pellet groups for the following 10 days. During this time the growth rates decreased moderately in the medium-sized fish group and reached a very low value of 1.74% in the largest fish. In contrast, the growth rates of the smallest size group still increased, compared to values obtained during the previous observation period.



**Figure 7.** Specific growth rates (SGR) of juvenile common dentex (*Dentex dentex*) for the screening period (SP) and the subsequent experiment, comparing dry and moist diets (EP 2a). Mean weights at day 1 ranged from 0.81-3.70 g and at day 50 from 11.60-18.68 g. During day 26-50 fish were fed either a commercial dry pellet (DP) or a self-prepared moist pellet (MP). Temperature: 22.8-25.7 °C.

Individual size and growth rates for the second experimental period 2b (comparison of dry and moist diets) are summarised in Table 10. Due to loss of fishes, no growth rates for the dry pellet groups could be determined. The moist pellet fish were growing at low rates during the first week and increased their growth rate considerably during the second week (day 51-58). Since no results for the dry pellet group were available, the trial was terminated after these two weeks. Because of the lack of growth data on common dentex, the results are reported here for comparison later.

#### (b) Feeding

The variation of daily food consumption for 10 subsequent days is shown in Figure 8. During this period, a regular feeding regime with 4 daily feedings at satiation level was applied, except at day 33 and 40, when fish were weighted. Only 3 instead of 4 feedings were offered at these

Table 10: Experimental period 2. Mean weight (g), standard deviaton ( $\pm$  SD) and the specific growth rate (SGR, % bw/day) are given for 2 test groups (dublicate lay-out) comparing a commercial dry pellet (DP) with a moist pellet (MP). Fish of this trial were taken from the MP group of the experimental period 2a ( $\Rightarrow$  Table 9 ). n.d. = not determined due to incorrect previous measurement.

Days (date)	DP -small (x g $\pm$ SD)	DP - large (x g $\pm$ SD)	MP - small (x g $\pm$ SD)	MP - large (x g $\pm$ SD)
51 (20 Sep.)	10.55 $\pm$ 2.23 g	17.53 $\pm$ 3.88 g	10.38 $\pm$ 2.66 g	20.03 $\pm$ 5.15 g
58 (27 Sep.)	*	*	12.50 $\pm$ 3.44 g	23.00 $\pm$ 5.71 g
SGR			2.65 %	1.98 %
65 (4.10)	*	*	16.69 $\pm$ 4.15 g	28.55 $\pm$ 6.98 g
SGR			4.13 %	3.09 %

\* several fish of unknown weight were missing at the weighing day, probably due to predation (cat ?).

days. This resulted in a reduction of the daily food consumption, especially expressed at day 40. Since the second feeding of the day was cancelled, it is obvious that fish could not compensate this missing feeding during the remaining two feeding times of the day. Especially the medium sized fish expressed reduced food consumption at the first weighing day (33) and the follwing day, which was compensated during the following 3 days (34-37), when the food rations exceeded pre-weighing values. At the second weighing day (day 40) all three size classes showed a similar reduction in food consumption.

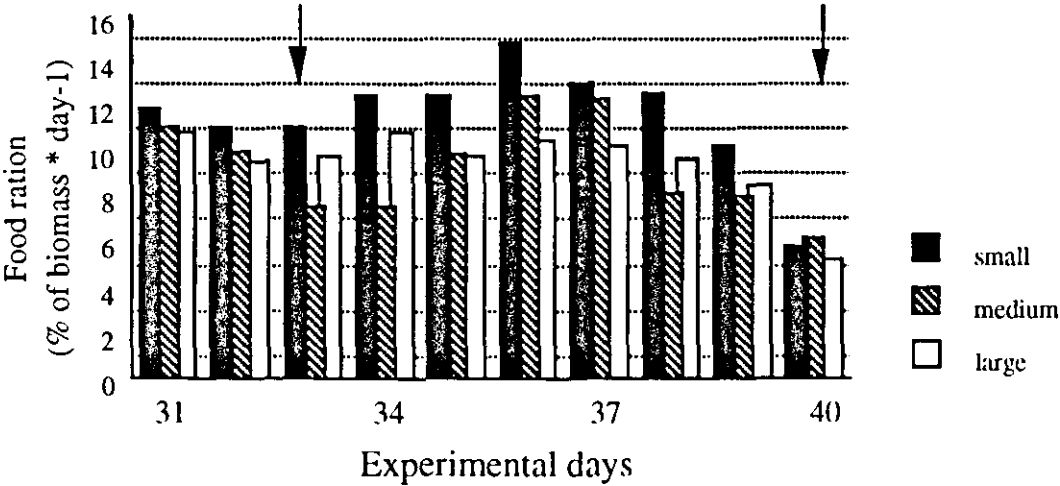
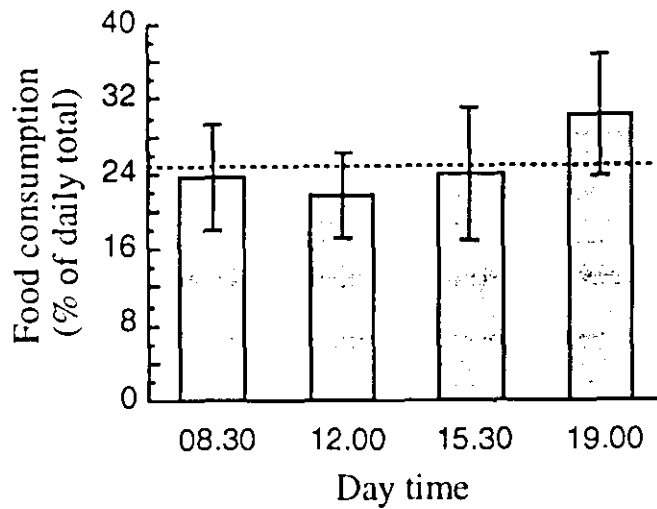


Figure 8. Fluctuations of appetite in 3 size classes of juvenile *Dentex dentex* (small: 5.5 g; medium: 8.3 g; large: 14.3 g) during a 10 days period. Values are given as moist pellet dry weight / biomass wet weight. Temperature: 24.4-25.6 °C. Arrows indicate weighing days.

The relative amount of food consumed at each single feeding is presented in Figure 9 as mean value of the 9 days observation period (except the last weighing day because of strongly reduced feeding). During the first 3 feedings of the day, fish consumed almost equal amounts of food (22-24%), whereas the last ration (19.00) was elevated (mean accounting for about 30% of the total daily consumption. However, a considerable variation between subsequent days occurred.



**Figure 9.** Mean food consumption of juvenile *Dentex dentex* (5.5-14.3 g) for 4 feedings during a daily feeding cycle. Fish were fed moist pellets offered by hand at satiation level. Data were sampled during a 9 day period (day 31-39) from 3 parallel tanks (n = 24). The broken line indicate the 25% line of equality level. Temperature: 24.4-25.6 °C.

### (c) Mortality

During the rearing of juveniles, a so called "normal mortality" due to not exactly defined, but mainly assumed reasons (poor adaptation to compound feeds, inhibited immune response of stressed specimens etc.) is commonly accepted in the present culture technique for this fish species. Usually, this mortality decreases when fish are on-growing and are becoming either adapted or more robust under given culture conditions. During the screening period (day 1-25) juveniles expressed "normal mortality" at a daily rate of 0.49% for the largest fish and at about 30-40% higher rates among the small and medium sized fish (0.73 % and 0.83%; Table 11).

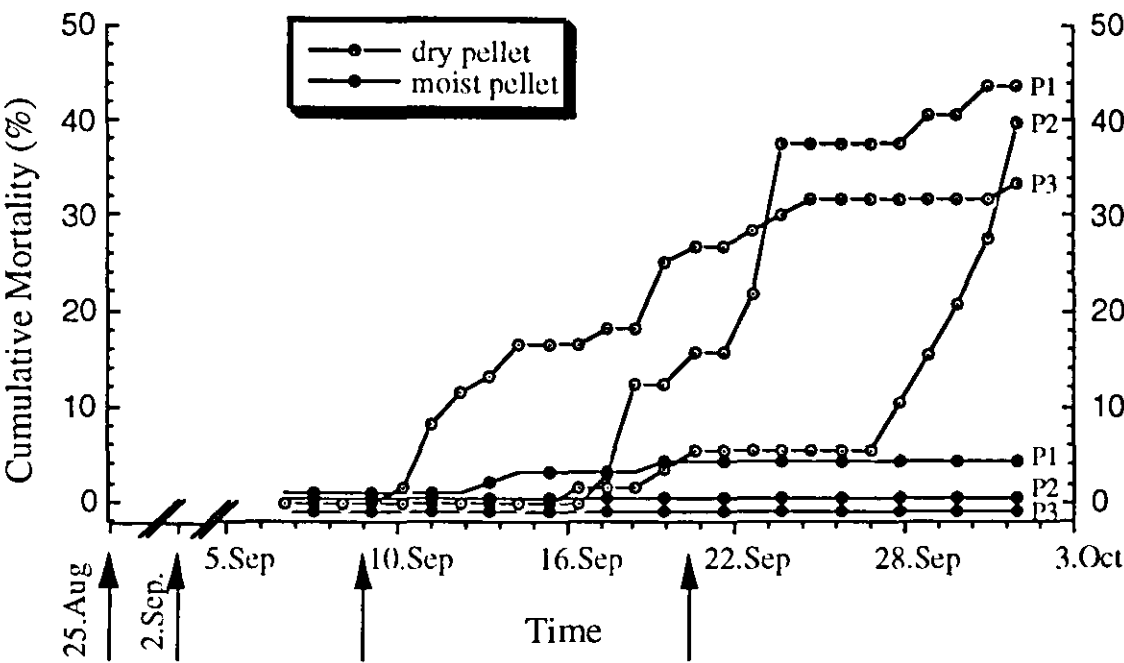
The development of an adequate moist pellet during this period as well as the total increase in fish size stabilized the overall condition of the fish and reduced the "normal mortality" at the beginning of the experimental period (EP) 2 to very low levels and almost no mortality was observed during the second week of this trial, although all fish were individually weighed each week, which was considered to cause severe stress. In the subsequent weeks 3 and 4, an infectious disease outbreak caused high mortalities in the 3 tanks fed on dry pellets only (0.52 % / d - 2.98 % / d). The relative low mortalities observed in the smallest size group of the moist diet fish were not caused by disease, but belonged to the previous kind of "normal mortality".

The cumulative mortalities of all tanks after the infection started are shown in Figure 10. Despite the time lack between the outbreak of the disease in the different size groups, a similar final mortality of 34% - 44% was observed for the 3 dry pellet tanks, whereas the moist pellet groups were not affected at all. The obvious relation between the kind of applied diet and disease susceptibility indicated a nutritional deficiency of the commercial bream-bass dry pellet concerning the nutritional requirements of common dentex juveniles.



**Table 11:** Daily mortality rates (% of initial tank number /day) for the screening period (EP 1) and experimental period 2a. The size of fish, small, medium, large (see Table 9) and the number (n) of fish at the start and the end of the experimental period are given for each tank. DP = Dry pellet; MP = Moist pellet. During the last time period (day 52-63), the MP fish had been already redistributed to repeat this experiment (EP 2a) (Table 10).

Time (days)						
Screening period						
(EP 1)	small	medium	large			
	n=223-148	n=222-150	n=61-54			
1-25	0.73	0.83	0.49			
<hr/>						
(EP 2)	DP 1	DP 2	DP 3	MP 1	MP 2	MP 3
	small	medium	large	small	medium	large
weeks (days)	n=60-38	n=32-18	n=58-35	n=90-86	n=31-31	n=90-82
1 (26-33)	0.00	0.42	0.00	0.56	0.56	0.00
2 (34-41)	0.00	0.00	0.00	0.16	0.00	0.00
3 (42-51)	0.52	2.59	1.25	0.35	0.00	0.00
4 (52-63)	2.08	0.97	2.98	-	-	-



**Figure 10.** Cumulative mortality (% of initial number) related to an infectious disease in juvenile common dentex. Total number of fish per tank varied between 31 and 90 at the beginning (25.8.92) of the trial. Arrows indicate the days of weighing of individual fish. Fish weight ranged from a mean of 3.73 g until 8.87 g at the 25. August for individual tanks respectively. P1-3 = Parallel treatments.

## 4.2 Broodstock response to transport and acclimation

### 4.2.1 Immediate effects of transportation

During transport fish behaved very calmly within the transport tank. Immediately after the transfer into the transport tank all fish stayed near the bottom without considerable swimming movements. During the routine oxygen checks (each hour initially and later each 2-4 hours) it was observed that the fish were distributed in the whole water column (1.2 m height), but still moving calmly, even when the tank was opened from the top and light entered suddenly. At arrival in Crete the brood fish were stocked in a relative flat (0.9 m water height) 10 m<sup>3</sup> square tank, together with two dentex of similar size which had been kept in this tank for more than a year.

During the transport of hatchery reared common dentex (EP 9), fish behaved similarly calmly as the wild brood fish during transport. The decline of oxygen in the transport tank was very constant during the whole transport (determined in 1 h-intervalls), indicating a constant oxygen consumption. When related to the biomass stocked, the measured decline of oxygen made up 190-240 mg O<sub>2</sub>/kg/h at a water temperature of 16-17 °C. Due to the high supersaturation of the water with oxygen (maximum = 280% O<sub>2</sub>), considerable amounts of oxygen have probably been diffused through the water surface into the atmosphere, and only a part was consumed by the fish.

### 4.2.2 Initial mortality and behaviour during adaptation

Although no mortality occurred during the first weeks after transport of wild brood fish (EP 3), which could be attributed to transportation stress, fish did not feed for a long period of 2-3 weeks and displayed a markedly nervous behaviour, which was manifested in burst swimming when disturbed from any activity outside the tank. Persons passing the tank, start of feeding etc. To initiate feeding in stressed broodstock fish, small live fishes (10-30 g) were given, which had been anaesthetized by a weak blow on the head in order to reduce their swimming capability. Unharmed juvenile seabream were not as easily accepted, but caused frightening behaviour among the many times larger dentex. However, even after a prolonged adaptation period and the onset of feeding, the wild brood fish expressed unnormal behavioural pattern, which are described in detail in the next chapter (Swim bladder stress syndrome).

The ability of hatchery reared brood fish to recover from transport and to adapt to new rearing conditions could not be determined, because of an accidental case of gas bubble disease in these fish immediately after arrival. This disease is described in more detail in the next paragraph.

### 4.2.3 Disease outbreaks in brood fish

#### (a) *Gas-bubble disease*

After transfer into a new installed broodstock tank (40-48 hours), a mortality of 2 fish was recorded. At the same time, about 10 of the remaining 29 fishes were very dark coloured, expressed swollen eyeballs (exophthalmie) and/or clouding of the eye cornea. The caudal part of the dorsal fin (soft spines area) expressed many gas-filled bubbles, which indicated a gas bubble trauma in these fishes. The measurement of total gas pressure in the upper part of the water column revealed a total gas pressure of 110%. Since this tank was 1.5 m deep, and the

hydrostatic pressure below a water column of 1m increases at about 10% (from 1 to 1.1 bar), the gas carrying capacity increases to an equal amount. Therefore the water at the deepest part of the tank was gas saturated at about 100%. This explained why several fish did not show symptoms of gas bubble disease, some fish probably were swimming near the bottom of the tank, whereas the hardly affected fish stayed in the top part of the tank.

During the same day a degasing column was positioned at the waterinlet of this tank. After passing the degasing column (80 cm height, filled with plastic substrate) the total gas pressure was reduced to 100%. Although no more fish died during the next two weeks after the accident, several fish were blind and could not eat any more, thus died later on and the other fish started feeding after a rather long period.

The reason for supersaturation was found easily after the problem became obvious. A high pressure pump, which was installed to supply the new broodstockhouse with water was leaking and therefore sucking air. This surplus air was dissolved due to the high pressure in the water resulting in 110 % gas-saturated water. It should be mentioned here that another new species, the black seabream (*Spondylisoma cantharus*) was stocked in a similar tank with the same supersaturated water and seemed not to be affected at all. The black seabream is a more benthic oriented species spending more time in lower parts of the water column of the tank and therefore was probably exposed to lower levels of supersaturation. Fishfarmers in Italy and Greece reported that mass mortalities among adult common dentex were observed due to supersaturation, when other aquaculture species such as seabass and gilthead seabream were not affected (pers. communication). From these observations, a high susceptibility of the common dentex to gas bubble disease can be assumed.

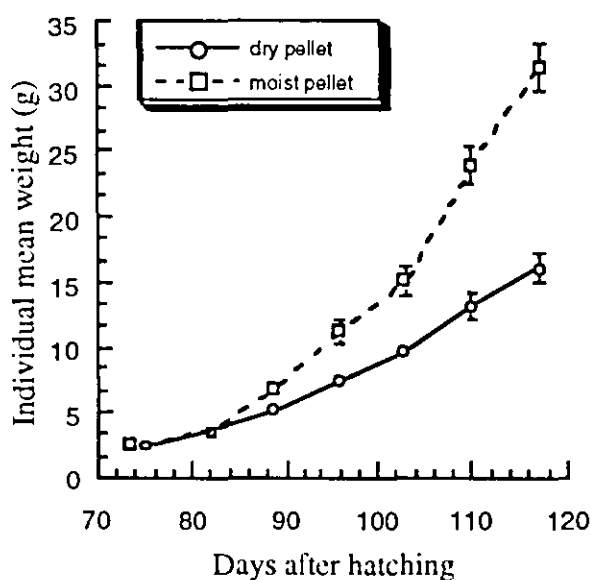
#### **(b) Swimbladder Stress Syndrome (SBSS)**

Several times broodstock fish displayed abnormal behaviour by swimming close at the water surface, preferably near the water inlet. Such a behaviour is typical for fish exposed to low oxygen conditions or in fish with problems of oxygen uptake due to gill damage or nitrite poisoning. Since no nitrite or other toxic agents occurred in the fresh seawater supplied here, and no accumulation of nitrogen compounds due to fish excretion should be expected to have occurred, considering the low stocking density (< 3 kg/kg) and high water exchange rates, an ectoparasitic or bacterial gill infection was first assumed to be the cause of this behaviour. However, antibacterial as well as antiparasitic treatments did not significantly improve the situation and after several weeks one of the largest fish, strongly expressing this abnormal behaviour, died. Examination of this fish did not show any damage of the gill tissues, nor any ectoparasites. All the inner organs displayed a rather normal size, shape and colour as known from healthy fish. Only the swimbladder seemed to be somewhat hyperinflated. Since no measurements of the inflated swimbladder were conducted nor comparable data from healthy fish were available, this observation was not proven. However, the constant swimming close to the surface, with the back even exposed to the air and the inability to swim in deeper parts of the tank, strongly indicated a positive buoyance as the reason for the observed behaviour. This syndrome was observed also in stressed individuals (social stress) during their second year of rearing, but not in juveniles during the first year of rearing. A swimbladder stress syndrome (SBSS) (Bagarinao and Kungvankl, 1986; Kolbeinshavn and Wallace, 1985; Johnson and Katavic, 1984), was finally assumed to be the reason for the abnormal behaviour, and in two cases also the death of the affected fish.

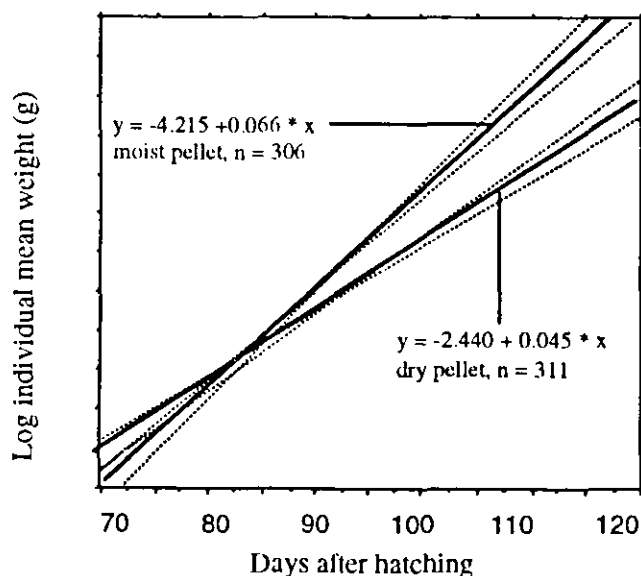
## 4.3 Growth, food conversion and agonistic behaviour in juveniles, fed on isocaloric pelleted moist or dry diets. Experimental period (EP) 5

### 4.3.1 Growth performance

Juvenile *Dentex dentex* fed on moist pellets showed a significantly better growth during the six weeks feeding trial than fish fed on dry pellets (Figures 11 & 12). Mean individual weight was attained in fish fed dry pellets 16.12 g (6-fold their weight), whereas fish fed on moist pellets attained more than 12 times their initial weight and reached a final mean weight of 31.3 g.



**Figure 11.** Growth (wet weight basis) of juvenile *Dentex dentex*, fed moist or dry pellets. Mean values and standard error from 3 replicates (pooled,  $n=45$ ) are shown. The trial lasted for 6 weeks (day 75-117 after hatching). Ambient conditions: Temperature = 24.4-26.2 °C, Salinity = 41-42 ppt, Oxygen = 4.8-5.9 mg/l.

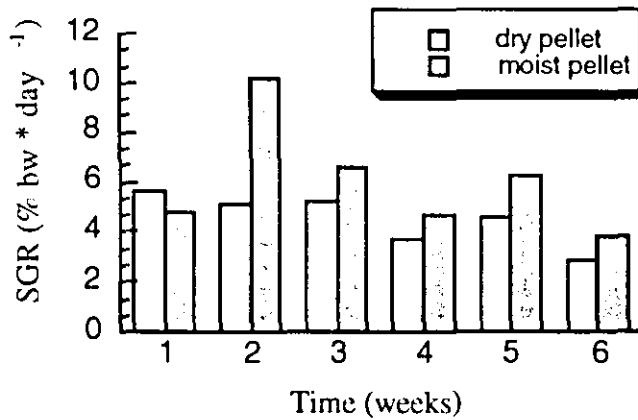


**Figure 12.** Regressions of logarithmic ( $\log_{10}$ ) fish weight (g) plotted against rearing time. Regression lines and Confidence bands ( $p \leq 0.01$ ) for the slopes ( $b$ ) of the two test groups, moist and dry pellets (MP, DP) are shown.  $r^2$  (MP) = 0.810,  $r^2$  (DP) = 0.793; standard error of  $b$  (MP) = 0.002,  $b$  (DP) = 0.001.

The main parameters of growth performance for this rearing trial are summarized in Table 12.

The comparison of the two growth curves concerning their statistical difference was conducted by linear regression analysis of logarithmic transformed fish weights. A comparison of the slopes (b) of the two regression lines (Figure 12) proved the significantly higher growth rate to be in the moist pellet group ( $p < 0.01$ , t-test).

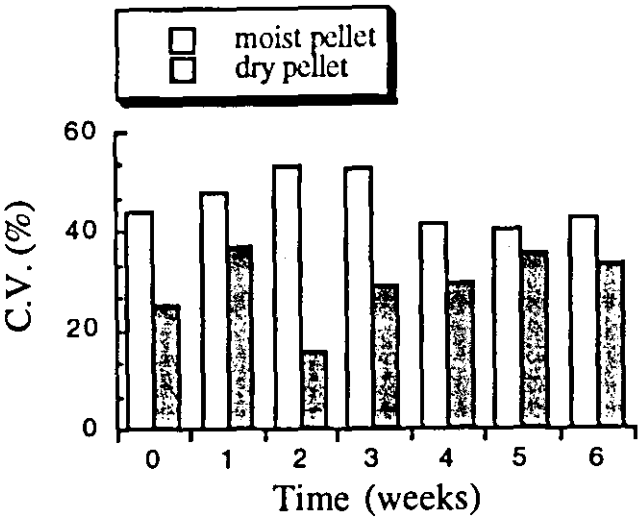
The corresponding specific growth rates (SGR, Figure 13) varied with time between 2.85 % (week 6) and 5.62 % (week 1) in the dry pellet group whereas fish fed on moist pellets, showed SGR values between 3.84 % (week 6) and 10.24 % (week 2). More common were SGR's between 5-6% in the dry pellet and 6-7% in the moist pellet groups. Parallel tanks, belonging to the same feeding group, expressed only small deviations from the means (not presented separately).



**Figure 13.** Specific growth rates (SGR expressed as % body weight per day) of juvenile *Dentex dentex* fed on dry or moist pellets. Mean weight ranged 2.43 g (week 1) and 16.2 g (week 6), dry pellet group and between 2.46 g and 31.3 g in the moist pellet group. Water temperature: 24.4-26.2 °C, Salinity: 41-42 ppt, max. stocking density: 2.62 kg/m<sup>3</sup>.

The Coefficient of Variation (C.V.) was considerably elevated in the moist pellet group, compared to fish fed the dry pellet diet throughout the entire experimental period (Figure 14). Fish relative size variability (C.V.) of the fast growing moist pellet fish showed an increasing trend during week 1-3, from 44% up to 53% and afterwards decreased during week 4-6 at a rate of 10-14%, even below initial values. The relative size variability in the dry pellet group almost remained at the same level (C.V.= 25-35%) throughout the entire experimental period, although considerable fluctuations occurred (week 2). Though all tanks were stocked randomly and mean weights corresponded very well (Table 12), the initial size distribution was already different between both diets, resulting in a higher standard deviation and C.V. value in fish fed on moist pellets (Figure 14).

The reason for this difference in size variability at the beginning of the trial (week 1) becomes obvious, when the respective size class distributions are plotted as bar chart (Figure 15). The initial stocking of the dry pellet group resulted in a very uniform distributed population, which



**Figure 14.** Changes in the Coefficient of Variation (%) (SD/arithmetic mean x 100) for juvenile *Dentex dentex* fed isocaloric dry or moist pellets. Calculations are based on weekly samples of 45 fish from each group (10-15% of total number).

was made up of only 3 size groups (size class = 1 g), whereas the moist pellet group included already individuals which could be assigned to 5 size groups and additionally expressed a significant positive skewness ( $p < 0.5$ ). During the 3 subsequent weeks, (week 2-4), the dry pellet fed populations displayed rather symmetric shaped size distributions. which coincided with the relative low values for skewness determined from the original data (not grouped into

**Table 12:** *Dentex dentex* rearing trials. Mean weight (g), standard deviation (SD) and Coefficient of Variation (CV) at the beginning of every week. Daily Feeding rate, Food conversion factor (FC, based on feed dry matter) and the Specific Growth Rate (SGR) for a feeding experiment, lasting 6 weeks and comparing a commercial dry pellet (Lansy 4, Artemia Systems, Ghent) and a moist pellet produced on site. All determinations are based on a sample size of  $n=45$ . bw = body wet weight.

Dry pellet						Moist pellet				
week	Mean weight x (g) ±SD	C.V. (%)	Feeding rate (% bw)	Food-Conv.	SGR (% bw/day)	Mean weight x (g)±SD	C.V. (%)	Feeding rate (% bw)	Food Conv.	SGR.. (% bw/day)
1	2.43 ±0.64	25.2	2.96	0.59	5.62	2.46 ±1.08	43.7	6.85	1.44	4.80
2	3.59 ±1.39	36.9	4.59	1.44	5.11	3.45 ±1.66	47.7	5.78	0.59	10.24
3	5.17 ±1.32	15.7	4.39	0.98	5.19	7.06 ±3.74	53.0	4.72	0.76	6.56
4	7.39 ±2.15	29.2	3.76	1.04	3.75	11.17 ±5.83	52.3	4.26	1.02	4.64
5	9.60 ±2.81	29.3	3.47	0.79	4.55	15.46 ±6.44	41.5	4.50	0.76	6.25
6	13.19 ±4.66	35.3	4.31	1.70	2.85	23.9 ±9.64	40.4	4.57	1.34	3.84
7	16.16 ±5.32	33.1				31.3 ±12.97	42.1			

size classes). During the last two weeks, the shape of the distribution curve was flattened, and for the first time so called "pre-growers" (particularly fast growing individuals) could be identified. This aspect is especially important with regard to social interactions and will be discussed later ( $\Rightarrow$  5.2). The first appearance of pre-growers during week 6 and 7 corresponded well with the increase of the coefficient of variation during the same period (Figure 14), and a significant positive skewness, indicating an asymmetry to the right size (larger fishes) of the distribution curve.

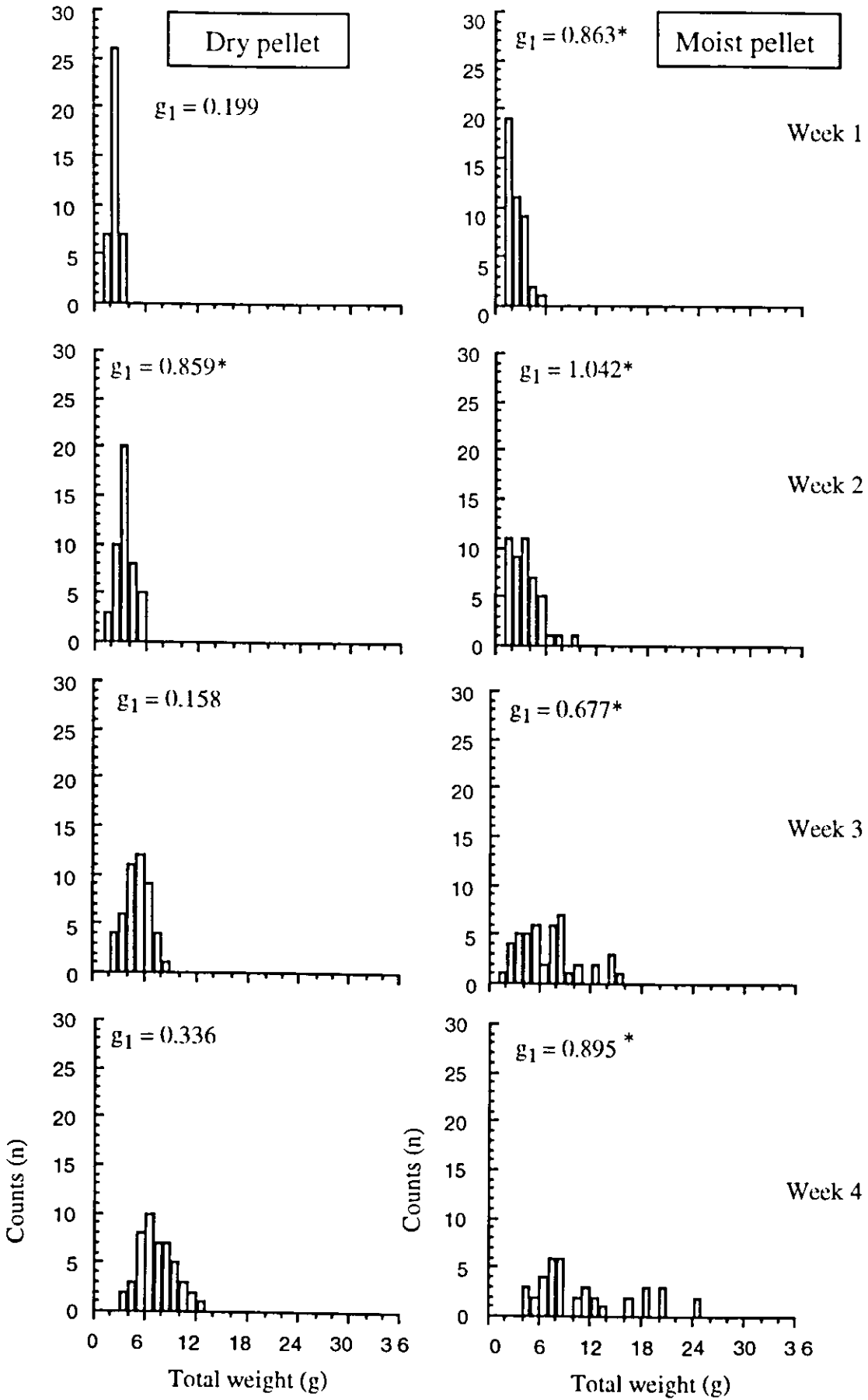
In contrast to the dry pellet fish, fish populations fed with the moist diet exhibited size distributions of a more flattened shape (platycurtic) with the first occurrence of "pre-growers" already during the second week (Figure 15). The obvious difference in symmetry between the two test groups at the beginning of the experiment (week 1) was strongly reflected by the determined skewness ( $g_1 = 0.199$  vs  $0.863$ ), with a significant positive skewness in the moist pellet group. However, note that during week 7 the lowest value for skewness ( $g_1 = 0.127$ ) was determined from a widely spread (biggest size range), but symmetrically shaped size distribution. The markedly larger range of size classes in the moist pellet group, when fish developed was probably caused by two effects: a) fish with a high growth potential, which grew considerably faster in the moist than in the dry pellet group and b) individuals with poor growth potential were surviving at a higher rate in the moist than in the dry pellet fish.

#### 4.3.2 Food Conversion

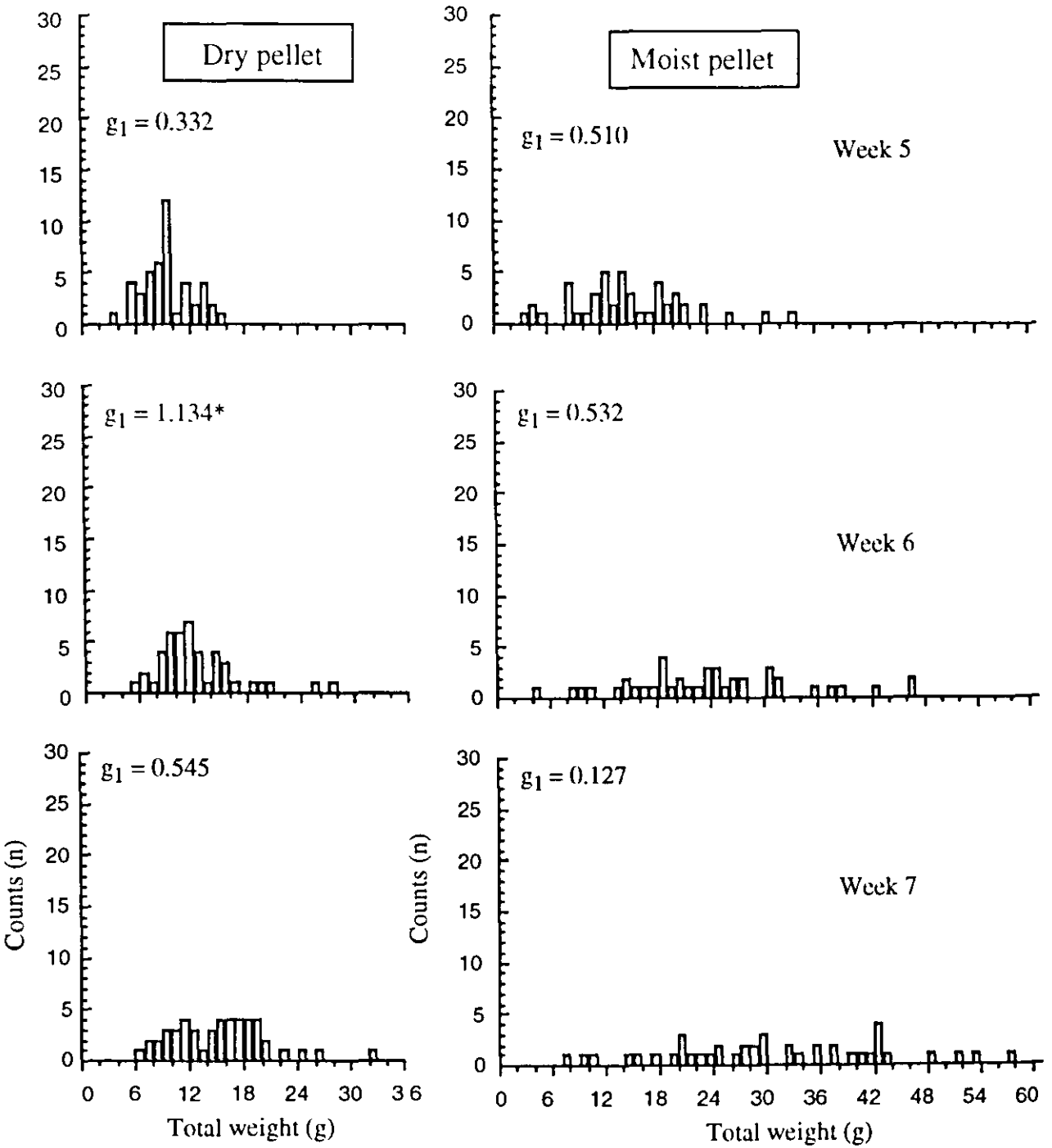
Daily feeding rates in the dry-pellet fish were about 1-3 % lower than feeding rates observed in moist-pellet fish (Table 12). This difference was even greater during the first two weeks, when feeding rates in the moist-pellet fish were high (6.85 %, 5.78 %), and at the same time, low in the dry-pellet fish (week 1 = 2.96 %). During the last 4 weeks of the experiment fish fed with moist pellets showed a very constant feeding rate of 4.26-4.78 % of their biomass per day. In this period, feeding rates of the dry pellet fish also varied little (3.47 - 4.39 %), but were still lower than in the moist-pellet fish.

Food conversion factors, calculated on a dry food base varied between 0.59 and 1.44 in fish fed the moist pellets, while these values ranged between 0.59 and 1.70 in the dry-pellet fish (Figure 16a). The best food conversion factors found in both diets, with F.C. = 0.59 were associated with the highest specific growth rates of 5.62 % in week 1 (dry pellet) and 10.24 % week 2 (moist pellet). The Food Conversion, calculated on a wet food base, however, gives a lower efficiency for the moist pellet group except during week 2 (Figure 16 b). This difference is explained by the highly different water content of the test diets. The Energy Conversion Rate (ECR) fluctuated between 10 and 25 MJ/kg biomass increase for the moist pellet and between 16 and 41 MJ/kg in the dry pellet (Figure 16c). The development of the ECR throughout the experiment was similar as described for the FC (based on a dry food base), since the test diets were almost isocaloric.

The Condition factor in the moist pellet fish increased continuously from a mean value of  $1.47 \pm 0.068$  during week 0 up to a  $CF = 1.67 \pm 0.060$  during week 6. The respective values of the dry pellet fish remained almost constant during the whole experimental period with mean CF values of 1.51-1.55. During the first 4 weeks of the experiment, the Condition factor did not differ significantly between fish of the two test groups, whereas during week 5 and 6, the moist pellet fish expressed a significantly increased condition factor compared to the dry pellet fed fish (Figure 17,  $t\text{-test}_{88/0.05}$ ).





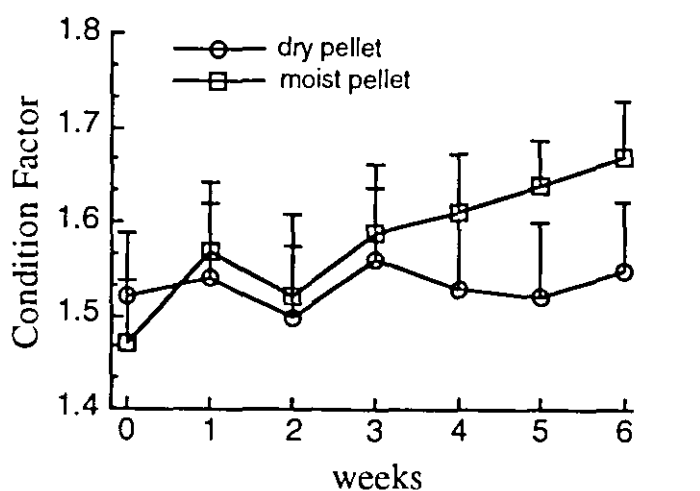


**Figure 15.** Size distribution in juvenile dentex (*Dentex dentex*) reared on dry or moist diets during a 6 weeks lasting period. Random samples of 45 fish (about 10% of tank population) were measured at the beginning of the trial and afterwards in weekly intervals (week 1-7). Size class = 1g; ( $g_1$ ) = skewness; (\*) = significantly positively skewed ( $p = 0.05$ ). The statistics (mean, standard deviation, coefficient of variation) for the weekly size distributions are given in Table 12.

**Table 13:** *Dentex dentex* rearing trial EP 5. Pellet dry weight, total food energy fed (KJ), biomass gain (g) and energy conversion as MJ per kg biomass increase for 6 experimental weeks are shown. Three replicate tanks (R1-R3) of each of the two test diets (dry pellet and moist pellet) were stocked with 150 juveniles (mean wet weight: week 1, DP = 2.43 g and MP = 2.46 g; week 6, DP = 16.16 g and MP = 31.30 g). The dry (Lansy 4 by Artemia Systems, Ghent) and moist pellet (MP 1993) energy contents were 21.21 and 23.35 MJ/kg respectively. The proximate composition of these diets is given in Table 6.

weeks	Dry pellet											
	R 1				R 2				R 3			
	Pellet (g dry)	KJ fed total	Biom. gain (g)	MJ/kg Biom. increase	Pellet (g dry)	KJ fed total	Biom. gain(g)	MJ/kg Biom. increase	Pellet (g dry)	KJ fed total	Biom- gain(g)	MJ/kg Biom. increase
1	121	2598	179	14.5	121	2598	156	16.4	126	2694	153	17.6
2	220	4704	94	50.0	220	4704	86	54.1	220	4704	275	17.1
3	271	5795	381	15.2	271	5795	186	31.2	295	6308	181	34.9
4	258	5534	181	30.5	258	5534	248	22.3	258	5534	242	22.8
5	299	6404	372	17.2	299	6404	382	16.7	295	6404	271	23.6
6	453	9699	374	25.9	453	9699	152	63.7	453	9699	285	34.0

weeks	Moist pellet											
	R 1				R 2				R 3			
	Pellet (g dry)	kJ fed total	Biom. gain(g)	MJ/kg Biom. increase	Pellet (g dry)	kJ fed total	Biom. gain(g)	MJ/kg Biom. increase	Pellet (g dry)	kJ fed total	Biom- gain (g)	MJ/kg Biom. increase
1	190	3992	134.0	29.8	191	2759	169	16.3	179	3763	131	28.7
2	268	5636	512.0	11.0	269	5654	514	11.0	244	5135	450	11.4
3	354	7451	582.0	12.8	344	7243	398	18.2	332	6988	585	11.9
4	441	9280	546.6	17.0	427	8984	617	14.5	396	8328	355	23.5
5	643	13511	863.5	15.6	633	13305	1011	13.2	561	11796	974	12.1
6	862	18120	1015.9	17.8	861	18111	509	35.5	801	16845	899	18.7



**Figure 17.** Condition Factor during a 6 weeks rearing trial feeding isocaloric moist and dry diets to juvenile dentex. Mean values and the upper standard deviation are given.  $n = 45$  for each mean.

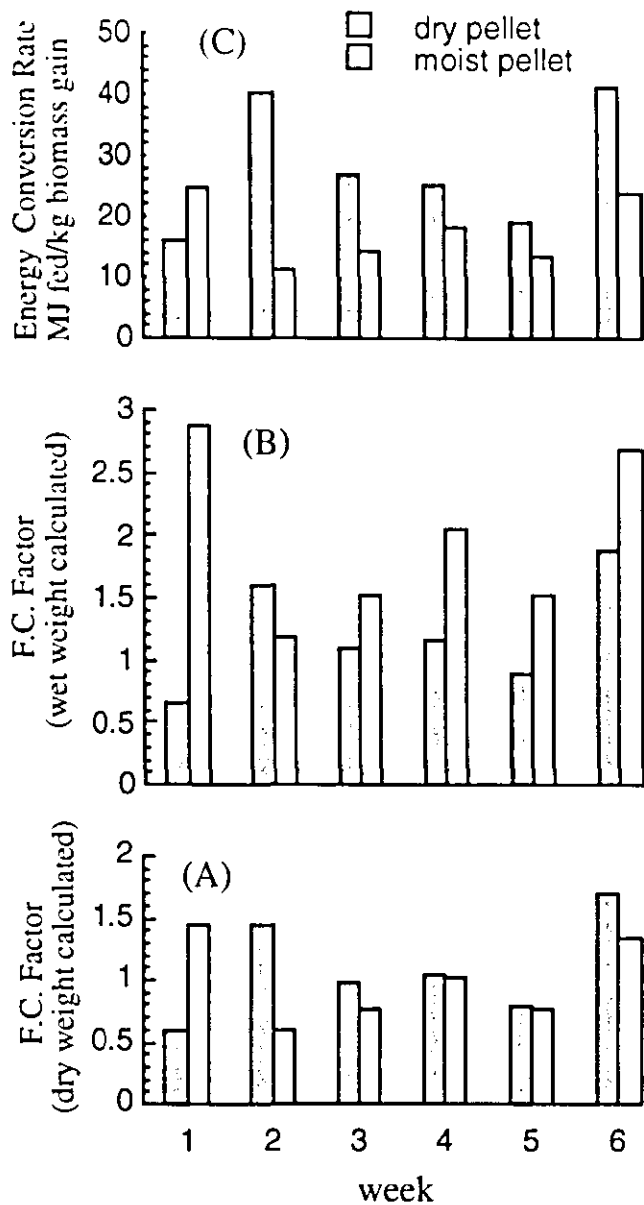
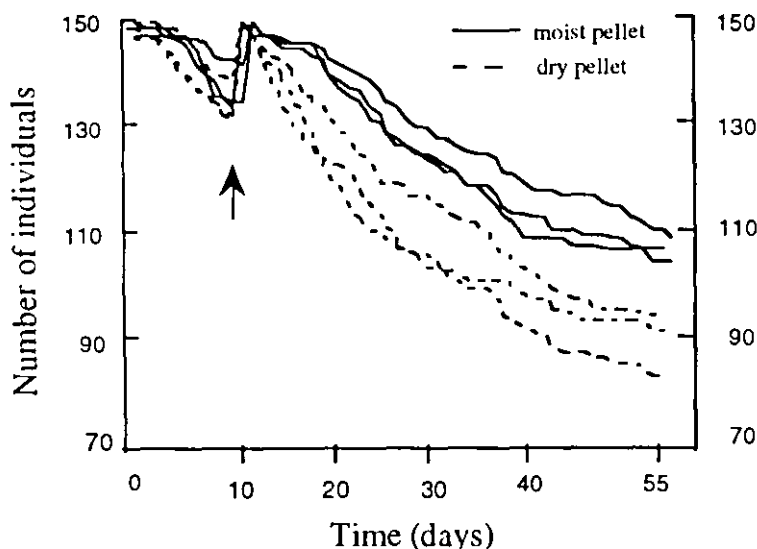


Figure 16. Food Conversion (FC) Factors, calculated on (A) a dry food and (B) a wet food base and Energy Conversion Factor (C) during a 6 weeks lasting rearing period. Juvenile dentex (2.4 3-31.3 g) were fed a dry or a moist diet (Lansy 4, moist pellet 94 ⇒ Table 6). The presented values are means, which are calculated from 3 parallel tanks (Table 13).

### 3 Mortality and Agonistic Behaviour

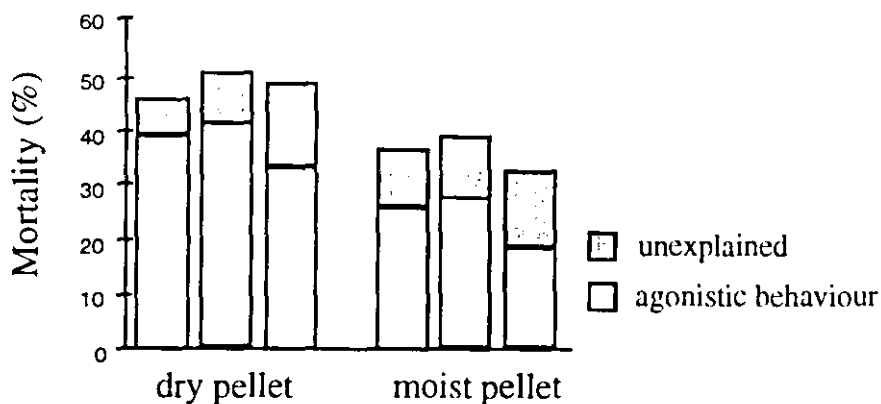
During the initial adaptation period of 12 days, when food in 3 tanks was changed from moist to dry pellets, the mean daily mortality was already higher in the dry pellet group compared to the moist pellet fish (0.85% vs 0.72%). This difference became significant during the experimental period, when the mean daily mortality decreased down to 0.64%/day in the moist pellet group and increased slightly (0.88%/day) in the dry pellet fish (Table 14). All three parallel tanks fed on dry pellets showed significantly lower survival rates with time than fish fed on moist pellets (Figure 18; Table 14). It is assumed that the mean total mortality of 48.4% (dry pellet) and 36.3% (moist pellet) observed over the entire experimental period (42 days)

was mainly due to biting attacks of tank mates (Figure 17) in both test groups. Mortality directly related to agonistic behaviour was significantly elevated in the dry pellet fish group reaching 78.5% of the total mortality compared to fish fed the moist pellets. In this group bitings were found to be the reason for death in only 64.8% of the dead fish (Table 14). Biting attacks were most commonly directed against the smallest individuals in the tank, as shown in figure 20. The remaining unexplained mortality was not different between both test groups (Table 14, Figure 19).



**Figure 18.** Survival of juvenile *Dentex dentex* fed a moist or a dry pellet. Each line represents a single fish tank. Day 1-12 = acclimation, day 13-55 = experimental period. The arrow indicates the restocking ( $n=150$ ) at day 12.

Agonistic behaviour was observed to occur in larger specimens by chasing and biting smaller individuals. The attack was almost always directed towards the tail of the subordinate fish. Bitten fish, with a bloody tail and staggering swimming movement, were attacked more frequently. The typical appearance of juveniles which died due to biting and due to not identified causes is shown in figure 6 ( $\Rightarrow$  3.17). The intensity of agonistic behaviour decreased during the experiment. No true cannibalism (ingestion of a complete tank mate) occurred during the observational period.

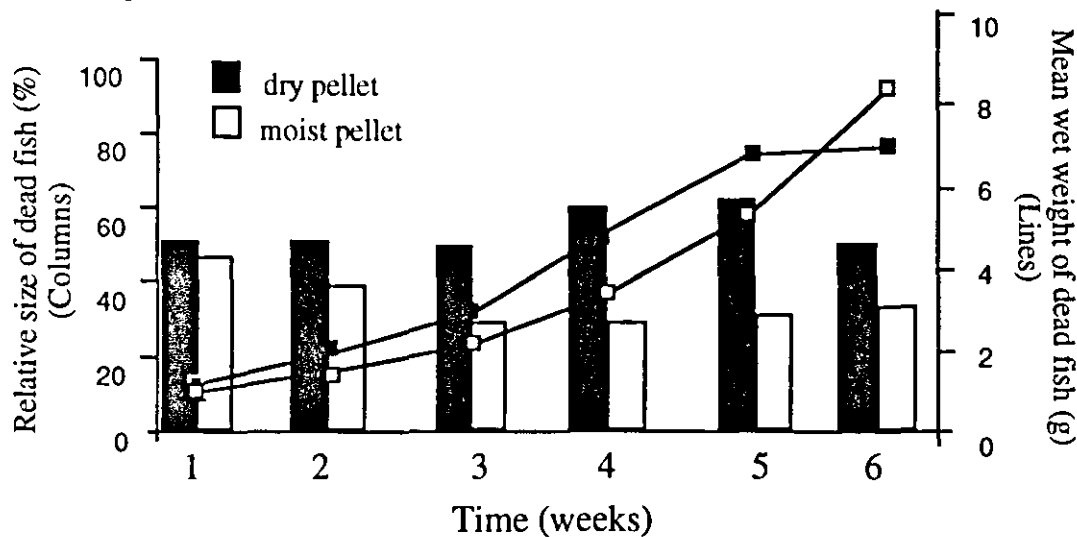


**Figure 19.** Total mortality rates, expressed as % of the initial number ( $n=150$  per replicate) of juvenile *Dentex dentex* for the entire experimental period (6 weeks), the part which was related to biting attacks (white columns) and the remaining unexplained mortality (black columns) is given for both test diets and each replicate tank.

**Table 14:** Mortalities presented as total number (n) and percentage (%) of the initial number (n=150) for each test tank in both feed groups (Replicate 1,2,3). The total mortality is separated in that related to agonistic behaviour and the remaining unexplained mortality. Mortalities are shown separate for the adaptation period (12 days) and the experimental period (43 days). Percentage per day is defined as total number of dead / initial number of fish stocked / time (days). Columns marked with \* are significantly different between both test groups ( $\text{Chi}^2_{(1/0.05)}$ ).

Mortality:	Total *		Agonistic behaviour.*		Unexplained mortality		Adaptation period		Experperimental period *	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	%/day	(n)	%/day
Dry pellet										
R1	69	46.0	59	85.5	10	14.5	17	0.94	52	0.80
R2	76	50.6	62	81.6	14	18.4	11	0.61	65	1.00
R3	73	48.7	50	68.5	23	31.5	18	1.00	55	0.85
Mean	72.6	48.4	57	78.5	15.7	21.5	15.3	0.85	57.3	0.88
Moist pellet										
R1	55	36.7	39	70.9	16	29.1	15	0.83	40	0.62
R2	59	39.3	42	66.4	17	28.8	16	0.89	43	0.67
R3	49	32.7	28	57.1	21	42.9	8	0.44	41	0.64
Mean	54.3	36.2	36.3	64.8	18	33.6	13	0.72	41.3	0.64

The wet weights of dead fish (which had died from frequent attacks) made up only 25-60% of the respective mean weight of the surviving fish. Although the absolute weight of fish died through bitings was quite similar for both test groups, the relative size was markedly lower in the moist pellet group (34.5% vs 53.8%) (Figure 20). Furthermore, the relative size of fish, which had died through biting, in the moist pellet group showed a decreasing tendency, 46% in week 1; 30-40% in weeks 3 to 6, while a slight increase of this size was observed in the dry pellet group (Figure 20). This difference in relative size of attacked fish becomes even more evident considering the much stronger size variation among moist pellet fish compared to dry pellet fish (Figure 14 & 15)

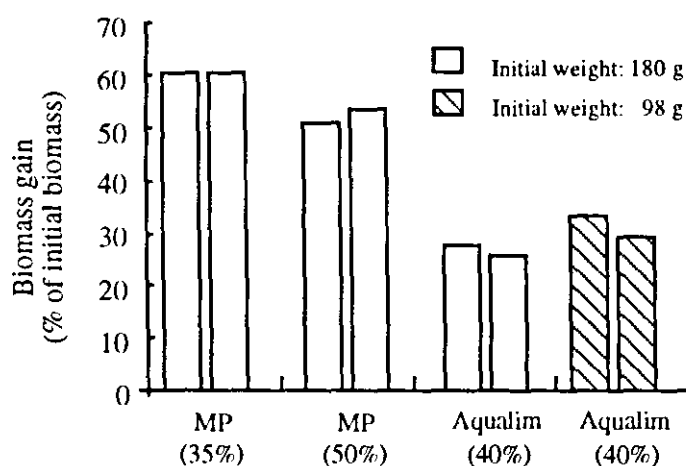


**Figure 20.** Change in size of dead individuals in juvenile *Dentex dentex* (lost due to biting attacks) during a 6-week rearing trial, when fish were reared on two different diets. Columns indicate the relative size of of fish lost by biting attacks, presented as percentage of the tank population mean weight. Lines indicate the mean wet weigh of dead individuals of both test groups (dry pellet, moist p.). N fluctuated, dependent to the weekly mortality rate between 5 and 30.

## 1.4 Effects of dietary moisture content in ongrowing fish

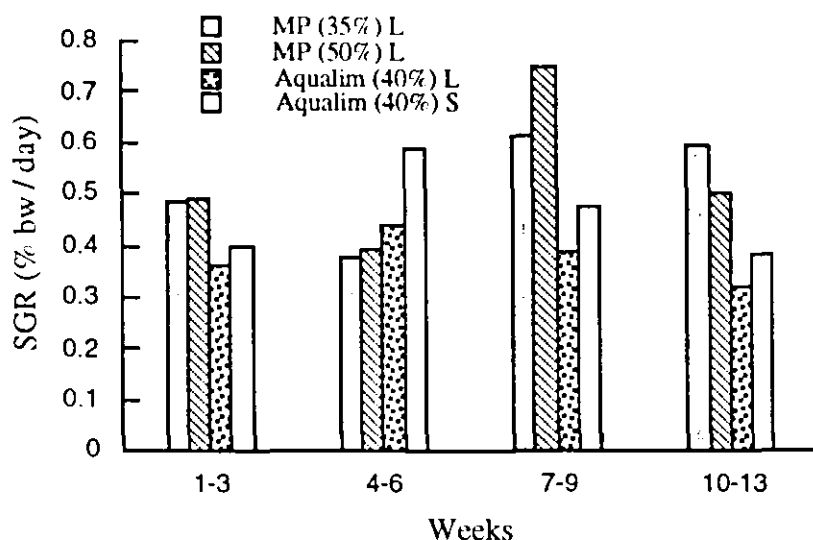
### 1.4.1 Growth performance and food conversion

All tanks fed with moist pellets (MP) increased their biomass at a rate of 50-60%, while fish fed on Aqualim pellets reached a considerable lower biomass gain of only about 30% during the entire rearing period (13 weeks) (Figure 21). This difference in biomass growth resulted from the combined effects of (a) elevated specific growth rates (Figure 22) and (b) lower mortality rates among the MP fish compared to the Aqualim pellet fed groups (Figure 27). Both groups fed with the MP containing 35% water achieved highest biomass growth rates with 60% respectively, while the MP with an elevated water content of 50% displayed only a slightly lower biomass growth. This difference was mainly due to the elevated mortality within the latter group, whereas specific growth rates of both groups were almost equal during the whole experimental period (Figure 22). The two size groups grown on the Aqualim pellet expressed a considerably lower (about 30%) biomass growth, compared to the moist pellet fed groups.



**Figure 21.** Biomass gain in common dentex (11-14 months old), when fed on moist diets of different composition (Moist pellet 94: Aqualim 2, for composition see Table 6), and water content (35, 40, 50%). Six tanks were initially stocked with 40 fish (mean weight 180 g) and two tanks with 55 fish (98 g mean weight). Temperature ranged from 15.8 to 25.1 °C, and feeding was twice a day ad libitum levels.

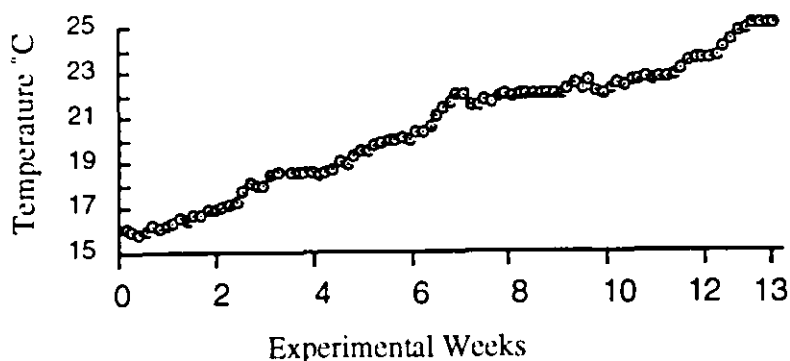
The development of specific growth rates is shown in Figure 22 for 4 subsequent time periods. During period 1 and 2 (weeks 1-6) both types of food resulted in almost similar specific growth rates of about 0.5 %. With increasing water temperature (Figure 23), the moist pellet fish expressed increased growth rates up to SGR's of 0.6-0.8% during the second period of the experiment (week 7-13), whereas the Aqualim fed fish expressed lower growth rates of 0.4-0.5% during the same period. During weeks 7-13 (June-July) water temperature had reached 20 - 25 °C, whereas during the first 6 weeks of this experiment, water temperature ranged only from 16 to 20 °C. Other water quality parameters, such as pH, salinity or oxygen content of the water did not change considerably during the experimental period (⇒ Materials: Table 3).



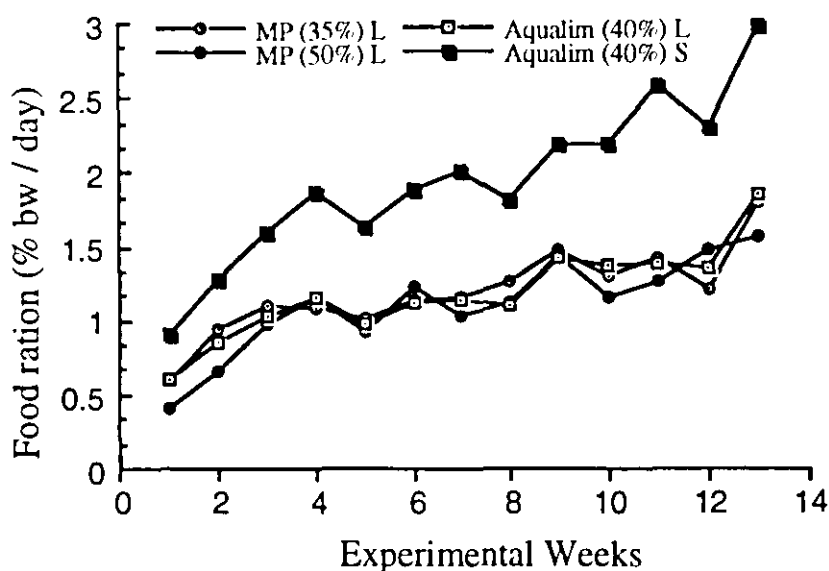
**Figure 22.** Specific growth rates (SGR) in on-growing common dentex (*Dentex dentex*), when fed on moist diets of different composition (Moist pellet 94 : Aqualim 2, for composition see Table 6) and water content (35, 40, 50%). Two initial size classes: large (L) = 180 g and small (S) = 98 g were employed.

The daily food ration (% bw) almost doubled in all test groups during the first 4 weeks of the experimental period. The larger fish (L) were consuming equal amounts of food dry matter, irrespective of food composition (MP/Aqualim) or water content (35 % / 50%), whereas the smaller fish consumed 60-80% higher food rations throughout the entire experiment. The relative food consumption increased rapidly during the first 4 weeks from about 0.5% to 1% for the larger fish and from 0.8 to 1.8% for the smaller sized fish. Parallel to this increase in food consumption, water temperature had increased from 16 to 19 °C, only. During the following 4 weeks (week 4-8), food rations did not increase further, although water temperature still increased continuously during this period. During weeks 9-13, food rations of all test groups were rising again, reaching values of 1.2 to 1.8% for the large fish. The development of food consumption in the smaller sized fish was similar (when compared with the larger fish), but almost at a 60-80% higher level. Thus, the latter group reached food ratios of 2.3-2.9% during the last 5 weeks of the experiment.

During the entire experiment, fish of the small size group fed on Aqualim pellet had highest Food Conversion factors (FC), which increased continuously from 2.8 during the first 3 weeks up to 5.9 during the last period (week 10-13) (Figure 25)^. This development was consistent with the elevation of water temperature and the increase in food consumption shown before. The large fish fed on the same diet expressed better food conversion rates, which ranged from FC = 1.8 to 4.4, but also increased continuously during the experiment. Both groups fed moist pellets, containing a medium and a high water content, expressed similar and much lower FC factors compared to the Aqualim fed fish during the whole experiment. These fluctuated between FC-1.3 and -3.2.



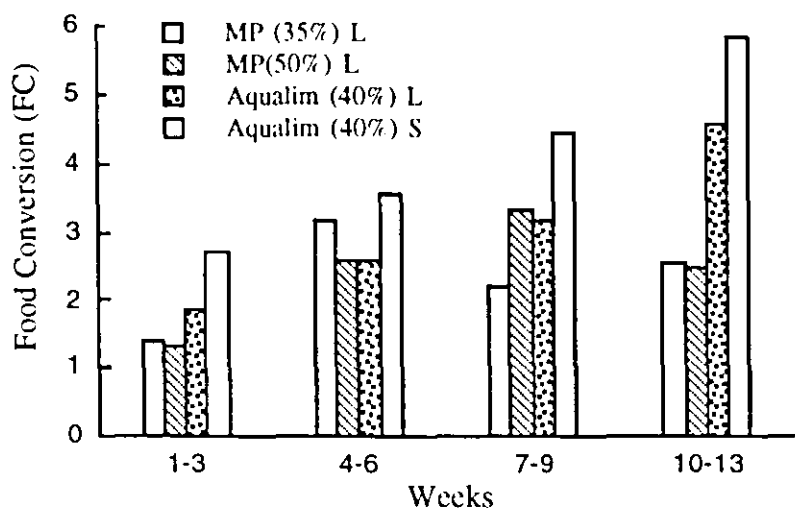
**Figure 23.** Increase of tank water temperature during experimental period 6. Corresponding to ambient conditions, the temperature increased from 16.1 °C at the beginning of April to 25.0 °C during mid of July.



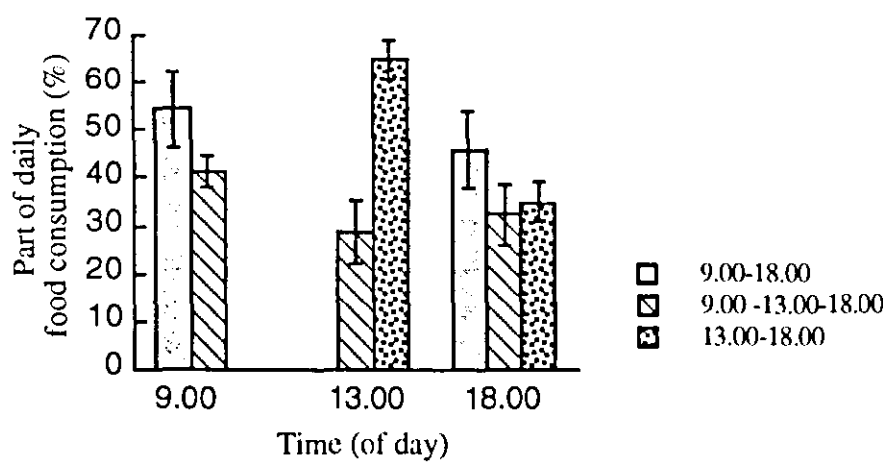
**Figure 24.** Food ration in 11-14 months old common dentex, fed on moist diets of different composition (Moist pellet 94 : Aqualim 2, Table 6) and water content (35, 40, 50%). Two initial size classes: large (L) = 180 g and small (S) = 98 g were employed. Fish were fed two times a day by hand ad libitum levels. Temperature increased from 16.1-25.0 °C during the experiment (Figure 23).

During this experimental period (EP 6), the main feeding regime was set at two feedings per day, one in the morning (9.00) and a second one in the evening (18.00). During week 8 this feeding regime was modified for 2 days, exploring if the increasing ambient water temperature would require a higher feeding frequency to satisfy fish's energy and nutrient requirements with regard to maximum growth rates. Another change of the regular feeding regime was related to problems with the water supply system during two subsequent days, when the morning feeding had to be shifted to a later time (about midday). Figure 26 shows the relative amounts of food, which had been consumed during each feeding of the day when different feeding regimes were applied. All three feeding regimes expressed the highest food consumption during the first feeding of the day (Figure 26). Changing morning-evening feeding (9.00 - 18.00) to midday-evening (12.00-18.00) increased the part of food consumed





**Figure 25.** Food conversion factor (FC, bases on food dry matter) of ongrowing common dentex fed on moist diets of different composition (Moist pellet 94 : Aqualim 2, Table 6) and water content (35, 40, 50%). Two initial size classes: large (L) = 180 g and small (S) = 98 g were employed.

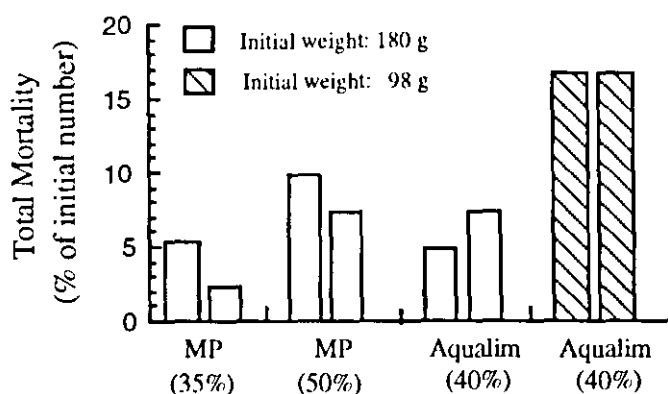


**Figure 26.** Changes in relative food consumption (% of daily consumed dry matter) of juveniles (age: 11-14 months) employing different feeding times and frequencies. Three different feeding regimes are compared: Two feedings per day (9.00-18.00 , n = 39 and 13.00-18.00, n = 8) and three feedings per day (9.00-13.00-18.00, n = 8). A moist diet was fed ad libitum levels. Temperature: 19 - 22°C; food ration: 1-2% bw / day.

at the first feeding from 55% to 63%. When feeding 3 times a day only 40% of the total daily food consumption was fed at the morning feeding. However, the shift of the morning feeding to midday feeding did not change the total amount of food consumption, whereas an increase in feeding frequency from 2 to 3 feedings, increased daily food intake at a rate of 29% at an ambient temperature of 22 °C.

#### 4.4.2 Mortality

The total mortality throughout the experiment ranged from 2.4 % in the MP (35% H<sub>2</sub>O) group to 16.7 % among the two test groups of smaller sized fish fed on Aqualim pellet (40% H<sub>2</sub>O) (Figure 27). The larger fish (180 g) reared on the Aqualim pellet (40% H<sub>2</sub>O) did not express significant different mortalities than equal-sized fish fed on the two types of moist pellets (MP). Although the two test groups fed with moist pellets of either 35% or 50% water content expressed a higher mortality in the latter group; this difference was not significant ( $\chi^2_{(1/0.05)}$ ). Since mortalities are reducing the biomass gain of a fish stock, the here observed differences in mortality rates between the test groups explain a part of the respective differences in biomass gain (Figure 21). Among the smaller size group, fed on Aqualim pellets, mainly the relatively high mortality was responsible for the strongly reduced biomass growth.



**Figure 27.** Total mortality rate in common dentex (11-14 months old) during the entire experimental period (13 weeks). Four test groups were fed moist diets of different composition (Moist pellet (MP) : Aqualim) and water content (35, 40, 50%). Initial stocking per tank was  $n=40$  for the larger fish and  $n=54$  for the smaller size group.

#### 4.4.3 Effects of feed moisture on water quality

Because feed composition and consistency may influence not only fish performance but also the dynamic processes associated with microbial water quality changes, it is important to identify the level and differences of soluble nitrogen compounds released by the fish during digestion. This may have drastic effects on system management because of the critical toxicity of unionized ammonia in high salinity (= high pH) waters.

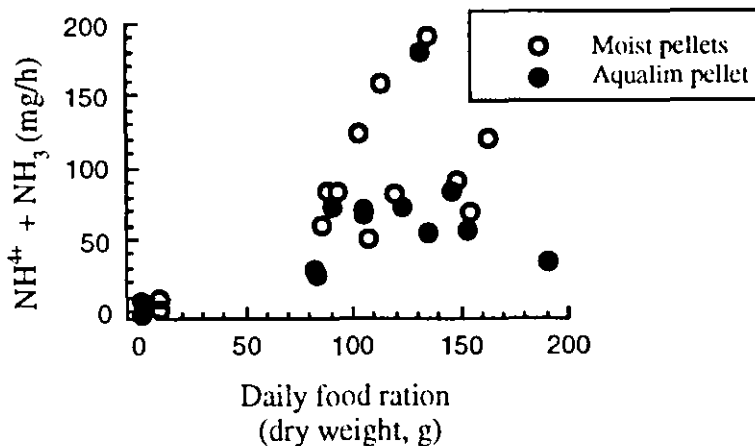
##### (a) Total ammonia ( $\text{NH}_4^+ + \text{NH}_3$ ) and Nitrite ( $\text{NO}_2$ )

The increase of total ammonia in fish tanks depends mainly on two processes, (a) the digestion of food protein and the resulting release of ammonia by fish through the gills and (b) the microbial degradation of food particles fallen to the tank bottom. Both processes are related to food composition and food stability. Therefore the increase of total ammonia was determined as the difference between tank water inlet and outlet concentrations for both types of food (Aqualim 2 : Moist pellet 94) included in this experiment. Values for both, total ammonia and nitrite were determined either during the morning (prior to feeding) or in the evening, 30 minutes after the last ration of the day was offered.

The water supply system did not contain any nitrite. Therefore, the determined level on the outlet represent truly released and accumulated amounts. Water samples, taken at the fish tank outlets contained nitrite at concentrations between 0 and 0.031 mg/L. Highest nitrite concentrations were measured during week 4, ranging from 0.015-0.031 mg/l. During later periods of the experiments nitrite values were at lower levels, and frequently below detection limit for the applied method.

Concentrations of total ammonia ( $\text{NH}_4^+ + \text{NH}_3$ ) in the tank supplying water fluctuated between 0.017 and 0.035 mg/l. Samples collected prior to feeding contained a mean concentration of 0.056 mg/l of total ammonia while determinations in the evening, 30 min. after the final ration of the day, displayed increased ammonia levels ( $\bar{x} = 0.084$  mg/l). The highest concentration of total ammonia determined from the outlet water of a fish tank was 0.229 mg/l.

The accumulation of total ammonia within the fish tanks increased from very low values of 2-6 mg/h during non-feeding periods up to values between 30 and 190 mg/h during feeding periods (Figure 28). Within this range of dry food matter, fed to single fish tanks, total ammonia production values fluctuated strongly, without displaying a clear trend. A comparison of the



**Figure 28.** Relationship between total ammonia production ( $\text{NH}_4^+ + \text{NH}_3$  tank outlet - tank inlet concentration (mg/l) \* flow rate (l/h)) and daily amount of food dry weight fed to a single tank. Measurements were conducted during week 6, 9 and 10. Stocking density ranged from 4 to 6 kg/ m<sup>3</sup> and water supply rate was 100%/h. 11-14 months old common dentex were fed at 1-2 % of biomass twice a day. Temperature: 16-25 °C.

types of pellet fed did not result in different values for both types of food. When ammonia accumulation rates were related to fish biomass the respective rates ranged from 6 - 23 mg/kg/h during feeding.

#### (b) Total Suspended Solids (TSS)

The feeding of moist diets is commonly related to high losses of soluble and particulate matter. This is mainly due to the reduced water stability of these diets, compared to pelleted dry diets. Although the here employed moist diets contained a binder (gelatin) to stabilize the consistence

of all test diets, a high turbidity was observed in the tank water after feeding started. Since moist pellets were fed at considerably larger pieces than dry pellets, fish did usually not swallow complete pellets. Biting into the soft moist pellet resulted in partial breakdown into smaller pieces, which did not attract fish and thus feeding activity on such particles was low. In order to estimate the particulate loss of moist diets into the rearing water during feeding, the amount of total suspended solids in the tank inlet and outlet water was determined for all test tanks.

With a mean of 33 mg/l TSS (n=3), the water supply line contained already a considerable amount of suspended solids. This solid load was dominated by inorganic particles, as the supply water was pumped directly from the sea, and filtered by a natural sand filter. Fish tanks contained total suspended solids in the range of 37.63 to 46.9 mg/l. Thus, the increase of TSS during feeding was within the range of 4.63 - 11.33 mg/l for different tanks. Due to the limited number of measurements and the high fluctuation of the solid load in flow-through systems, a calculation of suspended solid accumulation as well as the comparison between the different moist diets administered during this experimental period was not possible.

#### 4.5 Immunostimulation of juveniles by dietary intake of $\beta$ -1,3 / 1,6 glucans (Experimental period 8)

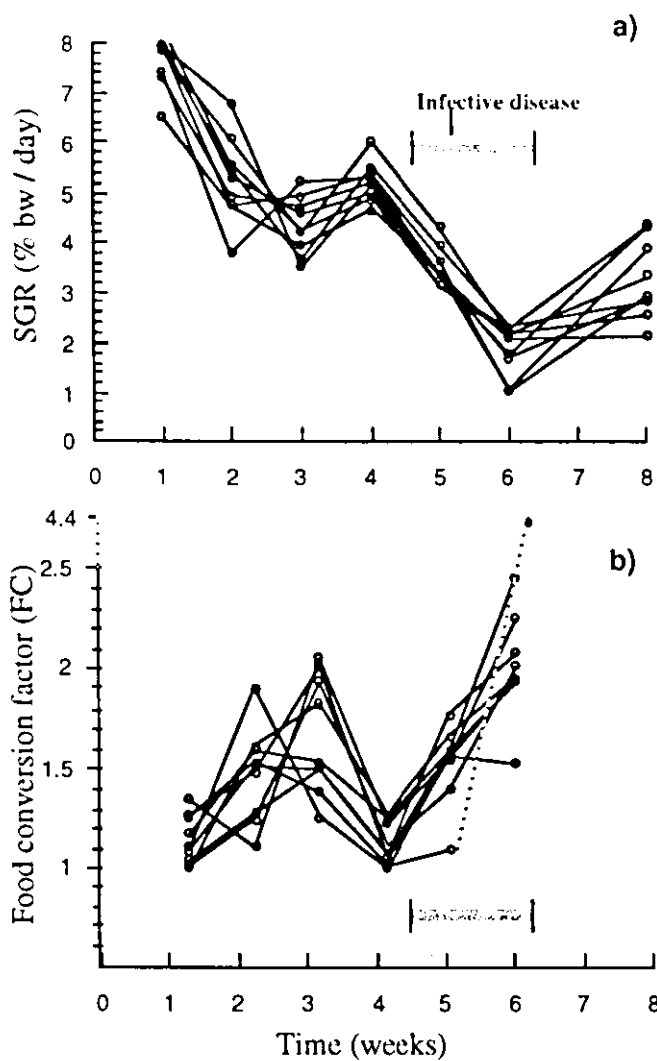
##### 4.5.1 Growth and food conversion

There were no significant differences in specific growth rate and food conversion between MacroGard, VitaStim and the control fish (7 weekly SGR's; triplicate tanks pooled). Neither

**Table 15:** (A) Comparison of Food Conversion factors and Specific growth rates between two groups of immunostimulant fed fish (MacroGard and VitaStim) and the control group. SGRs were size corrected in order to represent the SGR of a 10 g fish. (B) Comparison of food conversion factors determined during periods of disease and the disease-free time. Applied test: ANOVA; the F-ratio, Degrees of Freedom (DF), Number of values (N) and the probability of significance (P) are given for each parameter.

(A)	MacroGard	VitaStim	Control	F - ratio -(DF)	N	P
FC mean $\pm$ SD	1.51 $\pm$ 0.35	1.55 $\pm$ 0.35	1.64 $\pm$ .76	0.269 - (2)	51	0.765
SGR mean $\pm$ SD	4.34 $\pm$ 1.18	4.49 $\pm$ 0.92	4.43 $\pm$ 1.08	0.101 - (2)	60	0.904

(B)	Disease free period	Week 6 (Disease)	F - ratio -(DF)	N	P
FC mean $\pm$ SD	1.44 $\pm$ 0.33	2.28 $\pm$ 0.88	24.561 - (1)	52	<0.001
SGR mean $\pm$ SD	2.62 $\pm$ 0.71	4.73 $\pm$ 0.79	54.826 - (1)	61	<0.001



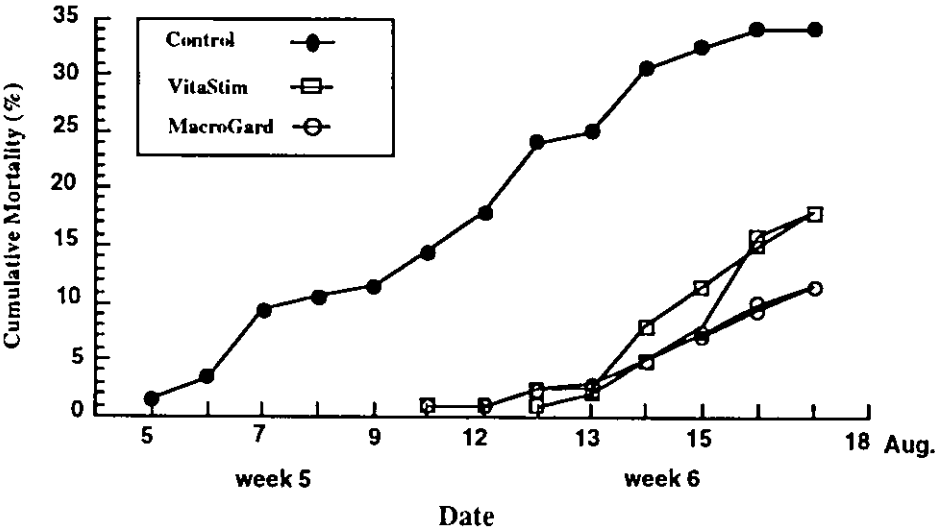
**Figure 29.** Changes in a) specific growth rate (SGR) and b) food conversion factor in juvenile common dentex during a 2 months rearing trial. All 9 test tanks are shown individually. The occurrence of an infective protozoic disease is indicated by the shaded bar. Note the coincidence of the decrease in SGR and increase in food conversion factor with the occurrence of an infective disease.

were there significant differences in these parameters between infected and non-infected tanks (week 5 + 6, triplicates pooled). During the first 4 weeks of the experiment, fish in all tanks expressed high specific growth rates of 4-8 % of body weight per day, followed by a sharp fall of growth rates during week 5 and 6, reaching lowest SGR values between 1 and 3 % in week 6. During week 8 a slight recovery of growth rates (SGR: 2-5 %) took place (Figure 29a). The reduced growth rates observed during week 5 and 6 were coincident with the occurrence of an infective disease in 5 of the 9 experimental tanks (3.16  $\Rightarrow$  Disease recognition and treatment). Comparing the growth rates of healthy tanks with infected ones, no significant differences were observed (not shown separately), but growth rates of all test tanks during the time of infection were significantly reduced compared to the remaining disease-free period (Table 15b). In order to make the specific growth rate, which is strongly size dependent, comparable between the different periods of the experiment, a correction of all SGRs determined was conducted. The corrected SGRs represent the growth rate of a 10 g unit fish ( $\Rightarrow$  3.20).

Stocking density increased considerably from initially 1.23 - 2.42 kg/m<sup>3</sup> to high final values of 6.49 to 14.4 kg/m<sup>3</sup>. The individual weight increased in the smaller fish from initially 2.5 g to 35 g after 8 weeks and during the same time interval the bigger fish increased from 4.2 g to 43g.

The food conversion factor (FC) during the first six weeks of the experiment ranged from 1.03- 2.47, and did not differ significantly between immunostimulated and control fish (Table 15a). Best food conversion efficiency was reached with values near FC = 1, between weeks 1 and 4 of the experiment and poorer food conversion efficiency was evident during week 6, exceeding mean FC values of 2.5 (Figure 29b). Infected tank populations did not have significantly lower FC values compared to non-infected tanks, but all test tanks expressed significantly lower FC values during the period when infection was present (week 6) compared to disease-free periods (Table 15b). Feed conversion factor was negatively correlated with specific growth rates: (SGR = 10.58 - 3.87 FC; r<sup>2</sup> = 0.58 , n = 53) (Figure 29).

4.5.2 Mortality

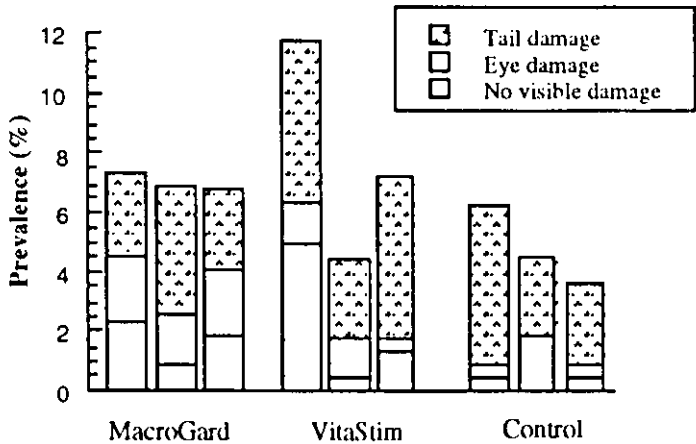


**Figure 30.** Cumulative mortality related to an infectious protozoan disease in juvenile common dentex. Total number of fish per tank was 200-220 at the beginning of the disease outbreak. 2 replicate tanks of MacroGard and VitaStim and 1 Control tank got infected. Mortalities stopped at 17. August.

Highest mortalities during the experiment were due to an infectious disease, which infected 5 of the 9 tanks during weeks 5 and 6. The infection was initially evident in one control tank, and 4 - 6 days later also affected two MacroGard and two VitaStim tanks. After 10 days, mortalities caused by disease stopped in all tanks nearly at the same time. Cumulative mortality in the control tank reached 30 %, in the two VitaStim replicates 15%, and in the two MacroGard replicates 10% (Figure 30). The fate of the infected fish varied between tanks with the highest ratio of survivors to mortalities in the MacroGard tanks followed by VitaStim; the lowest ratio of survivors was in the control tank (Table 16).

**Table 16:** Pattern of the infectious disease in the different test groups. Infected fish, the respective mortalities and recovered fish (survivors) as percentage of the initial tank population at the beginning of the disease (n = 205-217). M.G.=MacroGard, V.S.=VitaStim , C = Control, r 1,2,3 = replicate tanks

Test group	Infected fish	Mortality	Survivors	Ratio Survivors/Mortality
M.G. r 1	44.75	10.45	34.30	3.28
M.G. r 2	35.60	9.96	25.64	2.57
M.G. r 3	0.00	0.00	0.00	-
V.S. r 1	48.11	16.30	31.81	1.95
V.S. r 2	59.10	16.60	42.50	2.56
V.S. r 3	0.00	0.00	0.00	-
C. r 1	79.58	30.80	48.78	1.58
C. r 2	0.00	0.00	0.00	-
C. r 3	0.00	0.00	0.00	-

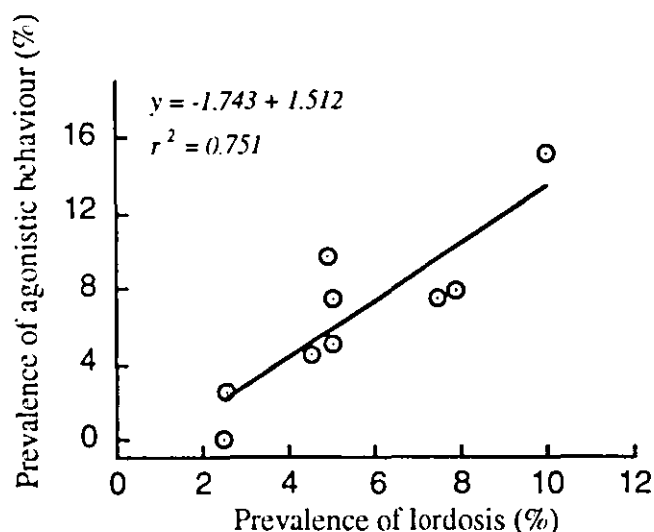


**Figure 31.** Mortality rates monitored during the whole experiment. Mortality due to agonistic behavior (tail damage), mortalities with severe eye damage and dead fish without external signs of damage are presented as % of the initial stocking (n=220) for individual tanks (triplicates).

Of the remaining mortalities (not caused by the infectious disease), most were due to tail damage caused by agonistic behaviour (Figure 31); other mortalities were related to eye damage and unknown causes. In the MacroGard group, however, mortalities were almost equally distributed between the three causes of mortality. The relatively high mortalities due to unidentified causes in VitaStim replicate 1 could be related to strong mucus production in this tank during a 3 day period in the second week of the experiment. An ectoparasitic infection was assumed, but no parasites could be found on dying fish during this period. A comparison of immunostimulated populations against control tanks conducted separately for each of the 3 categories of mortality did not show any significant statistical differences ( $\chi^2$  – test,  $p < 0.05$ ).

Among the survivors, which expressed a tail damage a relative high percentage of specimens with a deformed vertebral column (lordosis) was observed. Figure 32 shows the relationship between the prevalence of mortality caused by biting (tail damage) and individuals with a

lordosis. Since lordotic fish have a strongly reduced swimming capability they are more easily bitten by agonistic tank mates.



**Figure 32.** Positive relationship between the occurrence of Lordosis of the vertebral column and tail damage caused by agonistic behaviour in juvenile dentex. Each data point presents the results from a tank population estimated by a random sample of  $n = 40$  respectively.

#### 4.5.3 Blood parameters

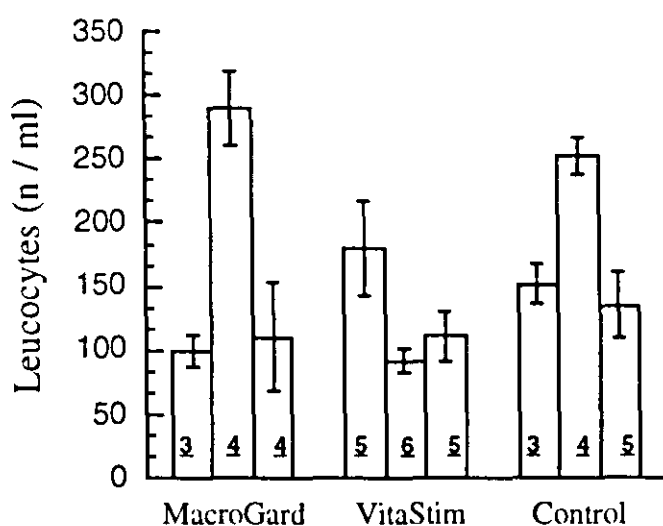
The measured blood parameters can be roughly separated into those giving a more general impression of fish condition (Hematocrit, Leucocrit), and those used as indicators for the activation of the non-specific immune response (NBT assay, Lysozyme activity, Plasma protein content).

**Table 17:** Indicators of nonspecific defense mechanisms in fish fed with diets supplemented by an immunostimulant (MacroGard or VitaStim) or a non-treated control diet over a two months period. Mean values and standard deviation are presented for each test group (replicates pooled,  $n = 13-17$ ). NBT = nitroblue tetrazolium assay. (\*) values differ significantly from control values in the same row (t - test,  $p = 0.05$ , two tailed,  $n = 30$ )

	MacroGard	VitaStim	Control
Hematocrit (%)	41.86 ( $\pm 7.72$ )	38.47 ( $\pm 6.45$ )	44.04 ( $\pm 8.51$ )
<i>Non-specific response indicator</i>			
NBT (extinction)	0.43 ( $\pm 0.075$ )	0.35* ( $\pm 0.087$ )	0.46 ( $\pm 0.093$ )
Lysozyme activity (%)	111.51 ( $\pm 6.65$ )	113.84 ( $\pm 9.58$ )	116.57 ( $\pm 9.85$ )
Total plasma protein (mg/ml)	54.95* ( $\pm 2.04$ )	53.21 ( $\pm 2.99$ )	50.57 ( $\pm 1.93$ )



Among these blood parameters the oxygen radical production (NBT assay) was significantly reduced in the VitaStim treated fish compared to control fish and the total plasma protein in MacroGard treated fish was significantly elevated. The other blood parameters, such as hematocrit, lysozyme activity and leucocyte number did not differ between the different treatments (Table 17). Leucocyte numbers (Figure 33) fluctuated considerably between 95.000 to 290.000/ml for single test tanks of even the same treatment. Within a tank population, variability was much lower and similar numbers of leucocytes were determined from fish of the same tank. The small number of samples obtained from this blood parameter was mainly related to analytical problems, particularly the relative fast clogging of blood cells. When blood cells start clogging, the number of counted, free suspended cells rapidly decreases. Therefore, only blood samples without signs of any clogging were included in figure 33.



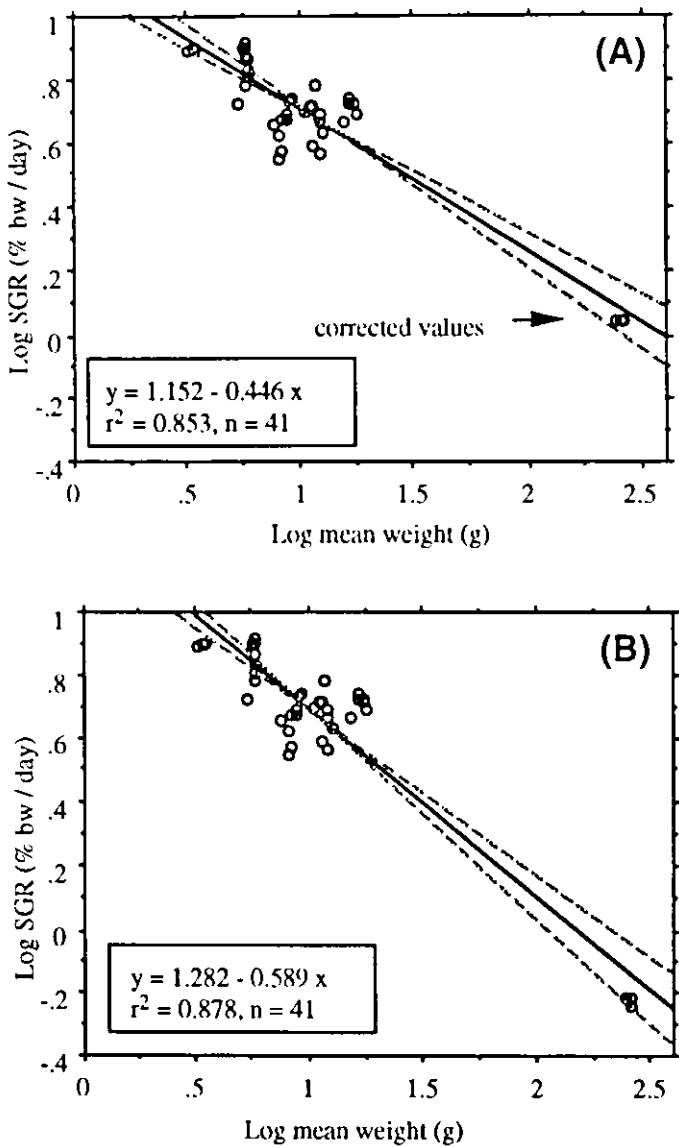
**Figure 33.** Variation in Leucocyte numbers between the replicate tanks of the different test groups. Mean values with standard deviation and the number of determinations (bottom of each column) are given.

## 4.6 Combined analysis of growth performance data from selected experiments

In the following chapter, data on growth performance and food consumption were compiled from all conducted rearing trials and analyzed in a way to establish general relationships, useful for easy comparison with results from other studies and the prediction of growth rates and food rations for this species.

### (a) Relationship between specific growth rate (SGR) and fish size

The decrease of growth rate with increasing individual size is a commonly known relationship in fish. Plotting the logarithm of growth rate (specific growth rate, SGR) against the logarithm of fish weight, over a range of weights, the growth rate can be seen to decrease following a linear function. The slope of this linear function is fairly uniform within a fish family (Brett, 1971) or even for species of different families (Jobling, 1983). Since Jobling (1983) predicted a

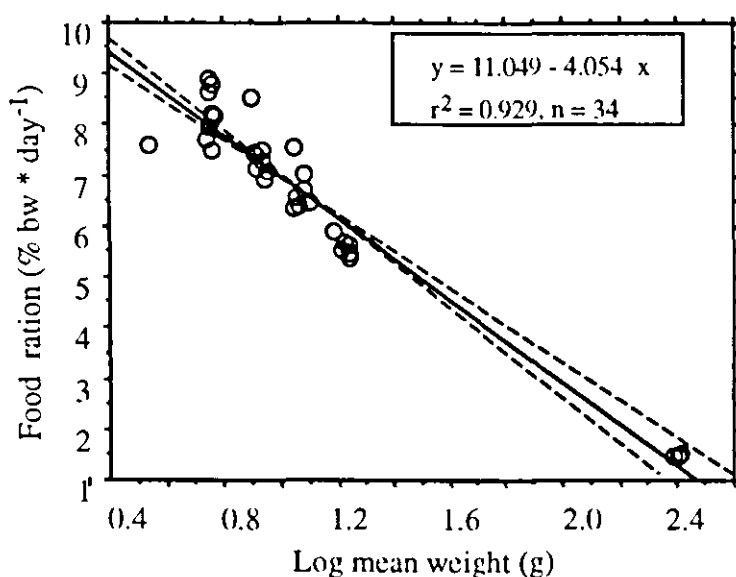


**Figure 34 (A+B).** Linear relationship between log specific growth rate and log mean weight in juvenile and ongrowing common dentex reared on moist pellets (MP 94) at a relative constant temperature of 23.7-25.1 °C. Figure B: regression for measured values; figure A: corrected specific growth rates for the larger fish (log 2.4 g). The regression lines and the 95% confidence bands (broken lines) for the slopes are given.

slope of about -0.4 to be generally applicable to fish species, the slope -0.589 determined by regression analysis of the specific growth rates of this study was assumed to be too steep (Figure 34 b). The steep slope of this regression was mainly caused by very low SGR's determined during the EP 6, when one year old fish were grown on diets of different moisture content (3 data points in the right part of the graph). Only one growth period of this experiments could be included in this regression because a similar temperature and diet were necessary to make specific growth rates comparable. Assuming reduced growth rates during this period (EP 6), an increase of growth rates from 0.67% (observed) near to the expected SGR of 1.1% was used for the determination of a corrected size dependent growth relation

(Figure 34a). Figure 34a shows the linear regression, which fitted best to the corrected SGR's, resulting in a slope of -0.4 to -0.5, which was predicted as typical for most fish species (see above). The hypothesis, that fish during the experimental period 6 did not express their full growth potential is supported also by other factors, which are discussed under 5.3.1 ( $\Rightarrow$  growth potential).

#### (b) Relationship between food ration and fish size



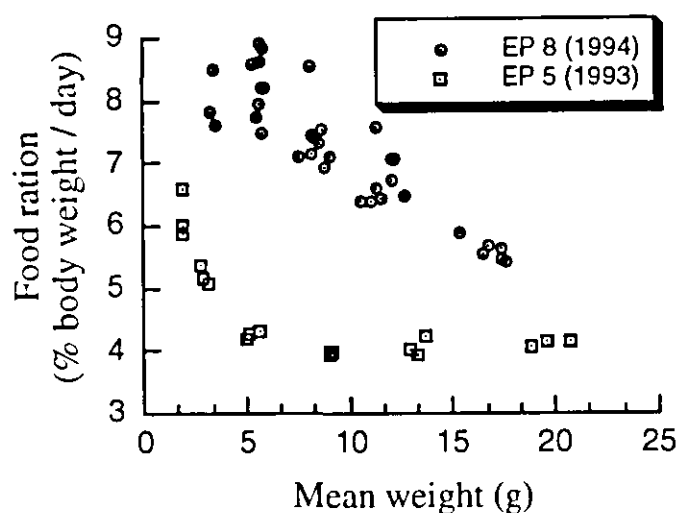
**Figure 35:** Linear relationship between food ration (% bw \* day<sup>-1</sup>) and log mean weight in juvenile and on-growing common dentex reared on moist pellets (MP 94) at a relatively constant temperature of 23.5-25.1 °C. The 95% confidence bands (broken lines) for the slope are given.

Correspondingly to the growth rate, the food ration was linearly decreasing with the logarithm of increasing body wet weight at a constant temperature (Figure 35). The linear regression, obtained from food rations determined during experimental period (EP) 6 and weeks 1-4 in EP 8 is highly significant. These experimental periods were selected for this analysis, since favourable culture conditions combined with similar physical (temperature) as well as nutritional conditions (Moist pellet 94) occurred. However, the comparison of food rations of juvenile fish reared during the periods EP 5 (1993) and EP 8 (1994) makes clear that food consumption varied considerably between the different rearing periods (Figure 36). The possible causes for the strongly increased food rations in 1994 are considered in the discussion chapter.

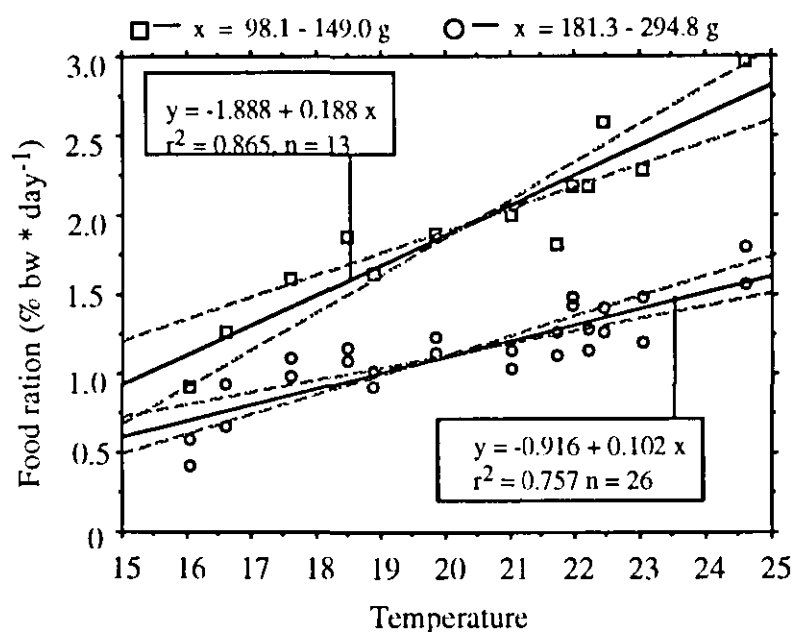
#### (c) Relationship between food ration, fish size and temperature

The increase of food ration with increasing temperature is described in Figure 37 for on-growing fishes of two different size classes. Both size classes show a linear increase of food ration within the temperature range of 16-25 °C, with the smaller fish expressing a more steep rise in food ration ( $b = 0.188$ ) than the larger fish ( $b = 0.102$ ). Caused by the size dependency of food ration in fish, as shown in Figure 35, smaller fish consumed more food relative to their body size than the larger ones. The data used for this analysis were taken during experimental

period 6. The food rations and temperatures shown here are weekly means, both based on daily measurements.



**Figure 35:** Comparison of size dependent food rations between the experimental periods (EP) 8 and 5.



**Figure 37:** Relationship between food ration and temperature in on-growing common dentex. Linear regressions are shown for two size classes (mean weight initial: 98.1 g - final: 149 g; initial: 181.3 g - final: 194.8 g). A high energetic pellet (21.2-22.5 MJ/kg) with high protein content (51.4 - 52.6 % protein) was fed twice a day ad libitum levels.

4.7      Morphometric characters and organo-somatic indices

This chapter deals with the analysis of morphometric characters and somatic indices in juvenile and adult common dentex and their changes with growth. The identification of distinct developmental stages can provide valuable information on the interaction of organisms with their environment. During growth, organisms develop in a way to fit best the actual life requirements. These do change particularly rapid and drastically in fish, animals that grow commonly from larvae of less than 1 mg to juveniles and adults of more than  $10^4$  -  $10^6$  mg body weight. This huge size range covered during a relative short developmental period, goes commonly ahead with habitat changes and fast changing food requirements. Especially the viscosity of water causes considerably scaling effects on hydrodynamics, mainly determining the efficiency of swimming.

The analysis was conducted by (a) the plot of each character as a percentage of the total length, (b) a linear regression model and (c) the change of several somatic indices with total length. The abbreviations used in this chapter are listed in Table 18.

**Table 18:** List of abbreviations for morphometric characters and somatic indices.

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Length and weight characters

TL	Total Length
SL	Standard Length
FL	Fork Length
HL	Head Length
PAL	Pre-Anal Length
POAL	Post-Anal Length
BD	Body Depth
ED	Eye Diameter
MOP	Maximum Mouth Opening
TW	Total Weight
KW	Karkass Weight

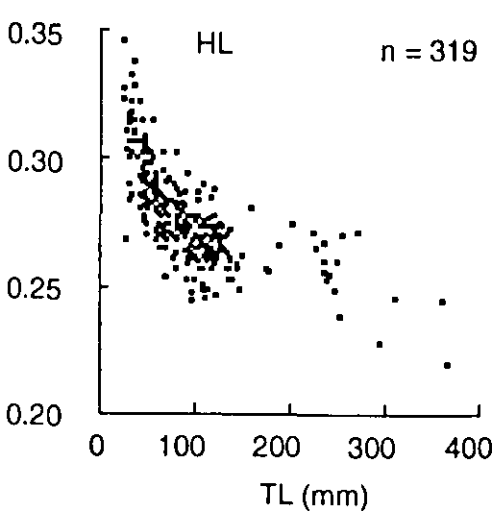
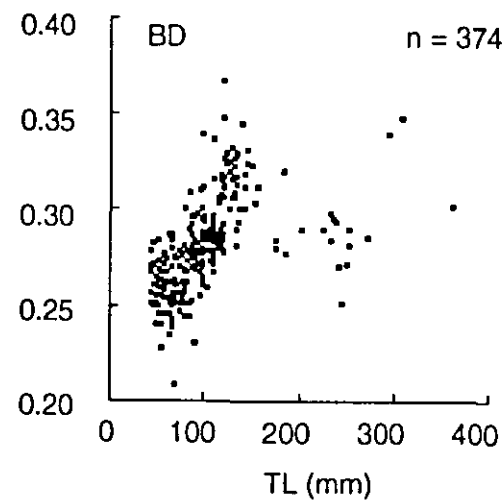
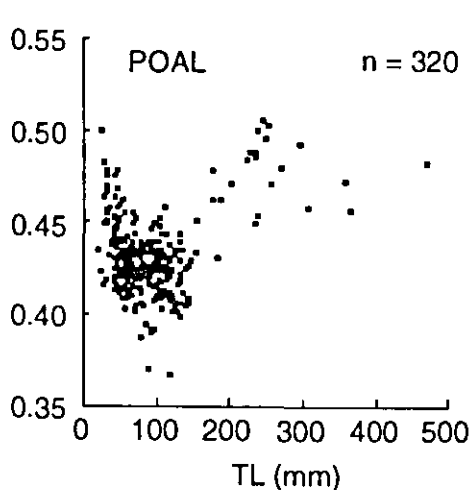
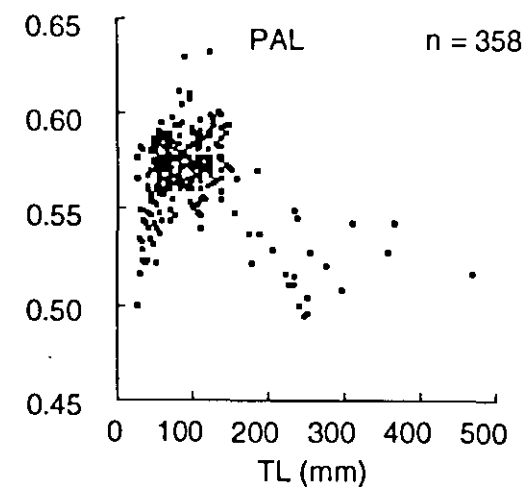
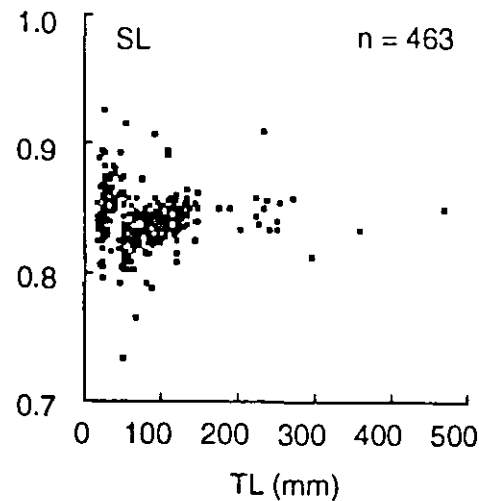
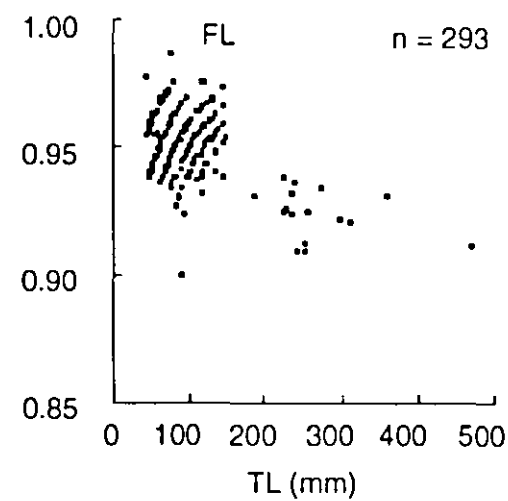
Somatic indices

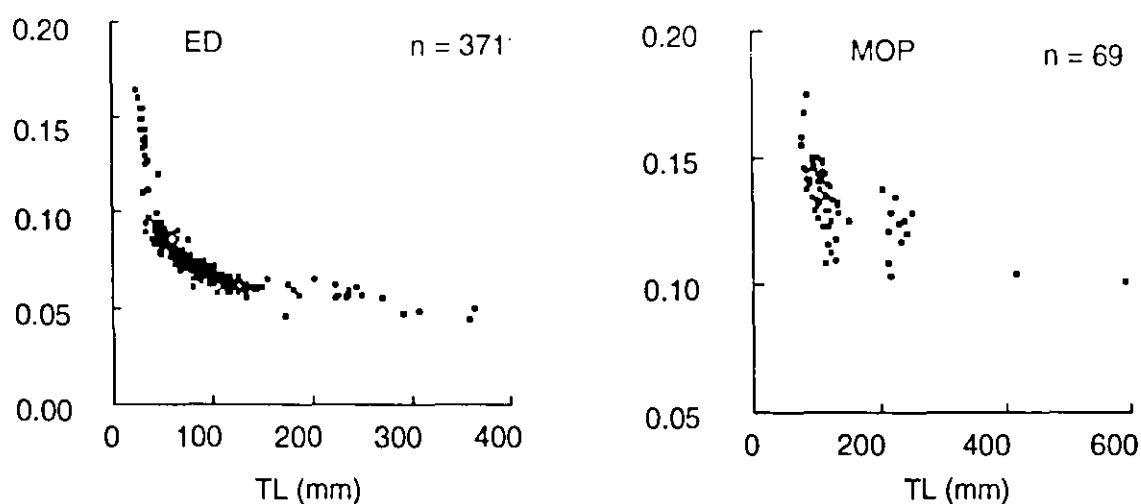
K	Condition Factor
GSI	Gonado Somatic Index
LSI	Liver Somatic Index
MFI	Mesenteric Fat Index
ILI	Intestine Length Index

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4.7.1      Changes in relative size of morphometric characters with growth

A graphical analysis of the relative size of each of the determined length characters (per-centage of total length) was conducted to determine differences in allometric growth. The analysis





**Figure 38.** Relative changes between eight metric characters (FL, SL, PAL, POAL, BD, HL, ED, MOP in mm) with growth (total length) in juvenile and adult common dentex (*Dentex dentex*), presented as part of total length (TL); (n) = number of fish measured. For abbreviations see table 18.

detects even small changes in growth patterns between the various morphometric characters and is therefore a useful tool to visualize the plasticity of these morphometric variables. Figure 38 shows the results for each of the length characters in fish between 12.3 and 470 mm total length. Most data have been collected from juveniles with a total length up to 170 mm; the larger sized fish are represented by smaller sample sizes.

The relationship between standard length (SL) and total length (TL) varied considerably in juveniles of about 20-100 mm TL (72% - 92%). This high variability of the relative SL is reduced in larger (older) specimens. No change in relative SL over the plotted size range can be identified from the plot. The relative fork length (FL) is slightly reduced in fish larger than 170 mm, compared to the overall relationship between the two length characteristics. This means that growth of the posterior part of the tail fin has increased in the larger size classes. General clustering patterns of several of the plotted data sets is considered to be incidental rather than reflecting true pattern.

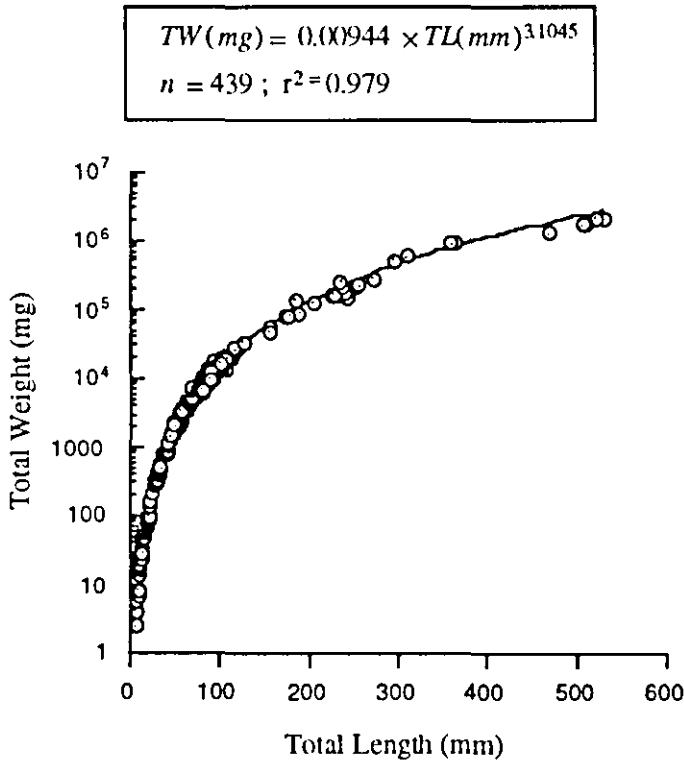
The plots of the relative Pre-anal-length (PAL) and the Post-anal-length (POAL) indicate their inverse relationship. While the relative PAL considerably increases in specimens of 12-80 mm (0.50% to 0.57%) and decreasing in larger fish of 170-470 mm (57% to 53%), the POAL shows almost constant values at 0.40% to 0.45% in the smaller fish and increases in the larger fish (from 0.45% to 0.50%). A decrease of the PAL and increase of the POAL are caused by a pronounced growth of the posterior part of the fish. This positive allometric growth accompanied by a change in the shape of the tail fin (see FL above), which occurs almost at the same developmental stage indicated an increase in the swimming ability of fish between about 100 and 200 mm TL.

The relative body depth (BD) increases considerably from 24-28% in fish of about 50 mm TL to about 30-35% in fish of 150-170 mm TL. In larger specimens a slight decrease seems to occur. However, only few data were available in this size range and the fluctuation of values is considerably, so that no precise trend can be extracted from this data-set.

The relative head length (HL) decreases exponentially with increasing total length and this development seems to continue in adult fish. Over the here examined size range, relative HL was reduced by about 20%. The graph shows that a high variability within one size group exists for this parameter. The relative eye diameter (ED) decreases considerably with increasing total length. Two break points of slope are visible in the plot, the first one at about 50 mm TL and the second one at about 150-170 mm TL. After the first break point, the slope of the (fitted) curve becomes reduced and after the second break point the slope is more reduced and the relationship between relative ED and total length almost linear. The degree of correlation between the relative ED and total length is the strongest one of all analyzed length characters. The relative size of the eye ball decreases from 15% in 12 mm fish to about 5% in 300-400 mm fish, which equals a total reduction of relative eye diameter by 66%.

4.7.2 Length-weight relationship in cultured fishes

The length-weight relationship was well described over a wide size range by a power function of the form  $y = a \cdot x^b$  shown in Figure 39. The data collected during different production trials and from several wild fish (mainly the largest specimens) give a highly significant correlation when fitted to a power model with an exponent  $b = 3.1045$ , which proves a positive allometry over the here examined size range.



**Figure 39:** Relationship between total weight and total length estimated from hatchery reared (< 300 mm TL) and wild (>300 mm TL) common dentex. Minimum size: 2.6 mg - 6.1 mm and maximal size: 530.6 mm - 4084 x 10<sup>6</sup> mg.



4.7.3 Development of morphometric characteristics in relation to length

For plotting all measured morphometric length variable against the respective total length, a log-log transformation was used, which leads to a more uniform distribution of the data over the entire investigated size range and therefore makes the detection of outliers (values not belonging to the estimated regression) easier (Figure 40). The results of the regression analysis shown in Table 19 were obtained from the respective non-transformed data-set, thus allowing a direct estimation of the dependent variables from the independent one (total length). The computed linear regression equations are of high significance for each length variable ( $F > 1000$ ,  $p > 0.99$ ). The strongest correlation were determined between FL, SL and the TL ( $r^2 = 0.999$ ), slightly reduced in PAL and POAL ( $r^2 = 0.90$ ), and the correlation between BD, HL, ED and TL expressed the lowest coefficients of correlation ( $r^2 = 0.976$ - $0.989$ ). The plot of the ED shows a group of about 20 fishes between 20 and 40 mm TL, which do not coincide with the linearity observed in the larger fish ( $> 40$  mm). These fish belong all to one single batch of measurement. The lowest correlation was found between MOP and TL ( $r^2 = 0.942$ ).

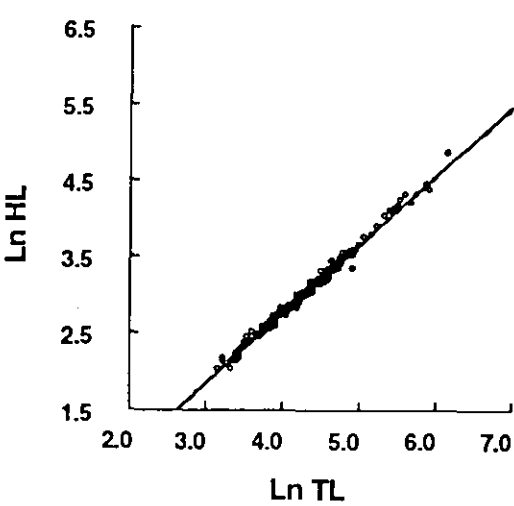
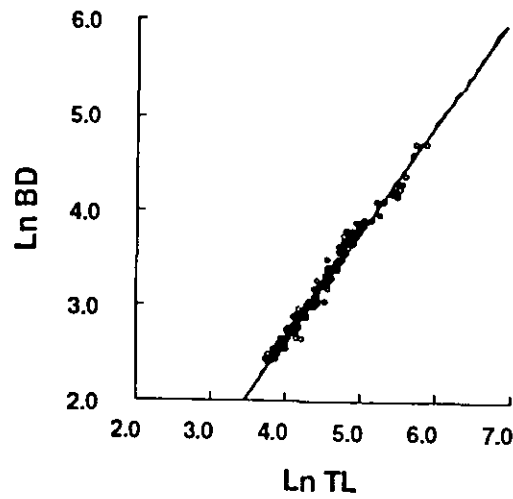
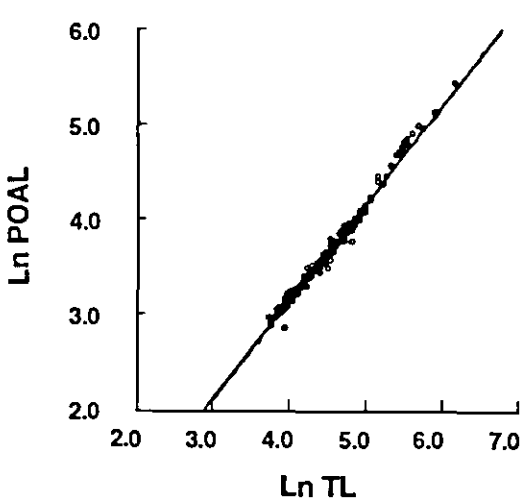
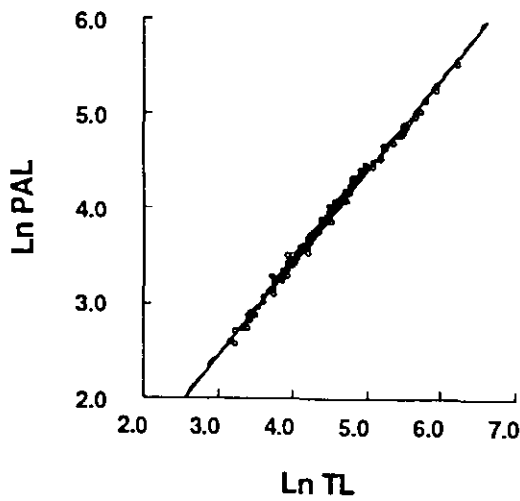
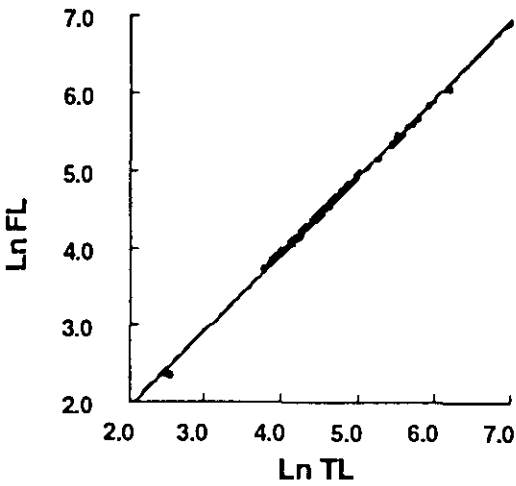
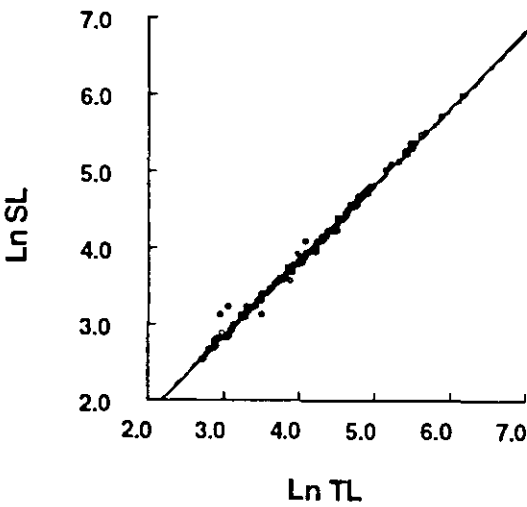
**Table 19:** Analysis of regression between the total length (TL) as independent variable and one of the measured length characters as dependent variable (column 1). The regression lines are characterized by their slope (b), the intercept (y), the standard error of estimate (SEE), the standard error of b (SE) and the squared coefficient of correlation  $r^2$ . N = number of measurements. For abbreviation see Table 18.

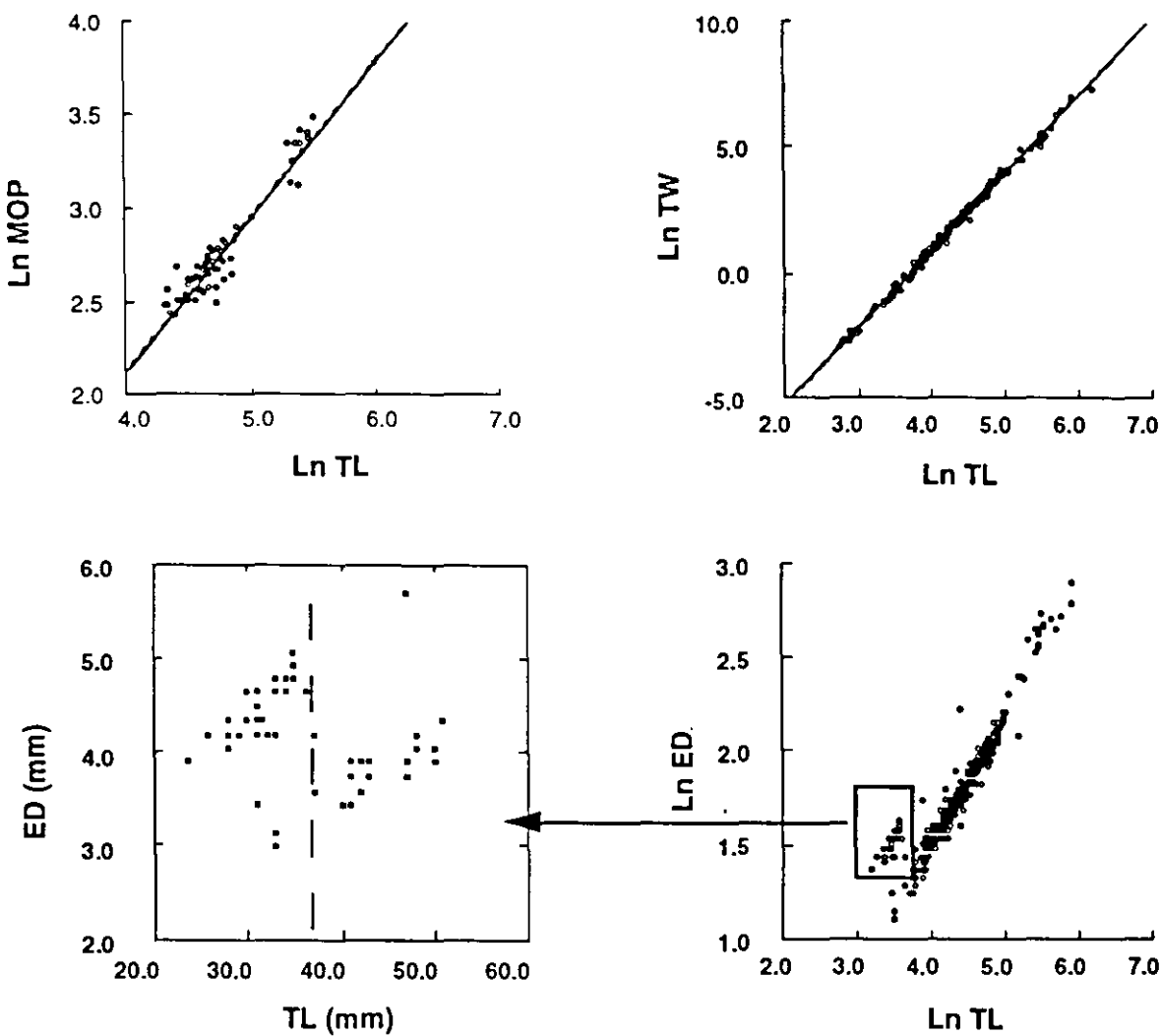
Character	Slope (b)	Intercept (y)	SEE	SE (b)	$r^2$	N
TL-SL	0.845	-0.288	1.690	0.002	0.999	463
TL-FL	0.924	2.525	1.810	0.002	0.999	293
TL-PAL	0.527	3.594	2.732	0.003	0.991	358
TL-POAL	0.481	-4.650	2.592	0.003	0.990	320
TL-HL	0.248	2.021	1.361	0.001	0.989	374
TL-BD	0.318	-3.221	2.338	0.003	0.976	319
TL-ED	0.044	2.264	0.739	0.001	0.989	371
TL-MOP	0.103	3.462	1.524	0.003	0.942	69

4.7.4 Organo-somatic-indices

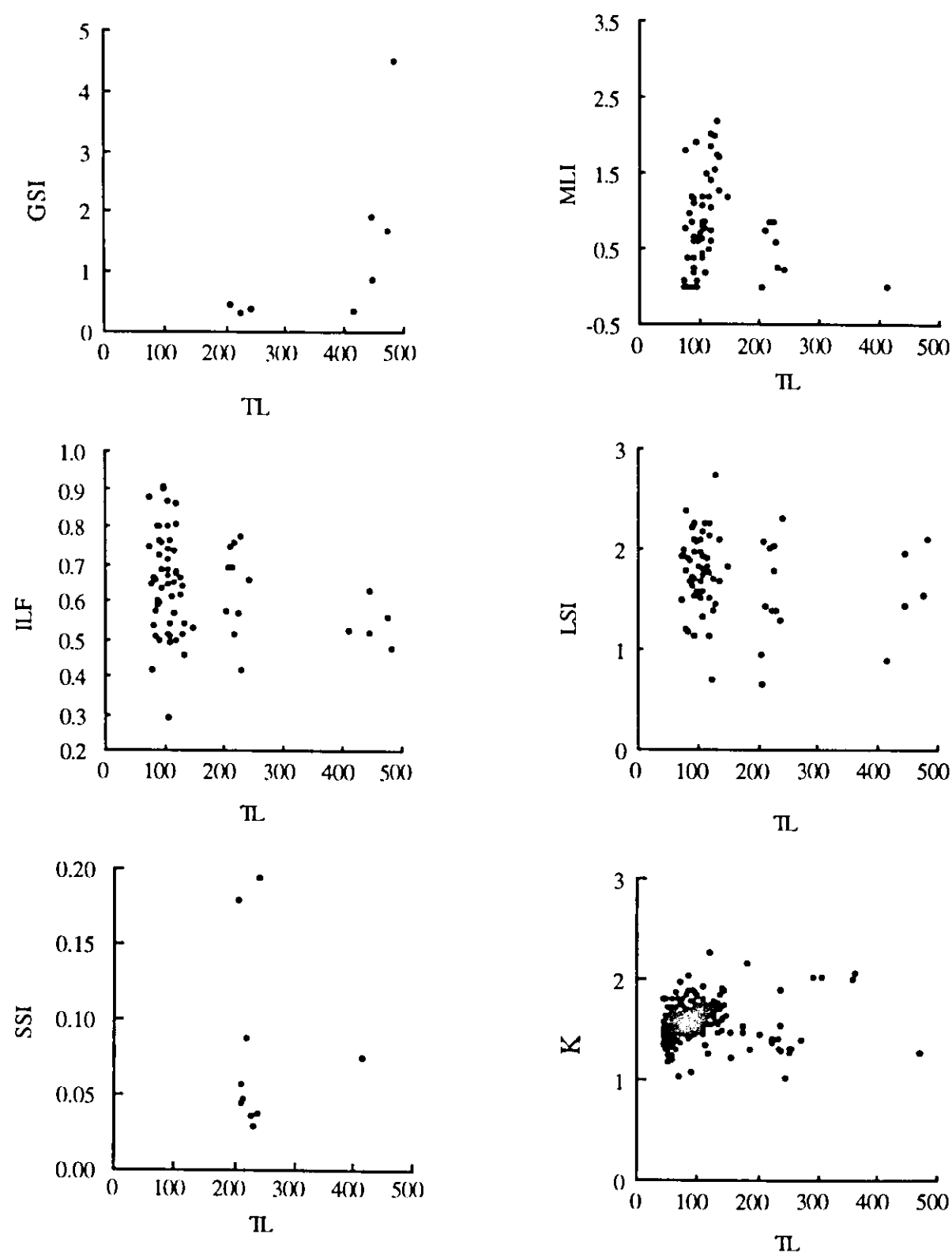
Somatic indices have been calculated from juveniles (age: 3-5 months) sampled during the experimental period No. 5. Additionally, about 1 year old juveniles and broodstock fish (experimental periods No. 6 & 9) have been examined to characterize the development of somatic indices with growth under culture conditions. The relationship between K, LSI, ILI, MFI, SSI, GSI and TL are shown in Figure 41. All somatic indices express a relatively high variation in fish of similar size. Except of the GSI, the mean values of all somatic indices did not change in the examined size range (100 to 500 mm TL). The few data available on the GSI indicate a rapid growth of gonadal tissue in fish from 400-500 mm TL. The mesenteric fat index (MFI) ranged from 0 to 2.4% of karkass weight. The relative length of the intestine (ILF) expresses a high variability from 40% to 90% of the total length with a mean of 60-70%. In larger fish this factor seems to be more constant. The liver somatic index ranges between 0.7% and 2.8% and seems not to be influenced by growth. The relative spleen weight (SSI) was determined in a few fish only (n=10). Thus, no detailed information on the development of this index with growth was possible. However, despite the small sample size, the high fluctuation

of this index in individuals of similar size becomes obvious. The SSI ranged from 0.3% - 2%. The Condition Factor (K), was a relatively constant somatic factor compared with the other somatic indices examined here. The condition factor values fluctuated between  $K = 1 - 2$  for the included size classes.





**Figure 40.** Linear regression between total length (mm), as independent variable and 8 metric length characters - SL; FL; PAL; POAL; BD; HL; ED; MO - (mm) and the total weight (g) as dependent variables, after logarithmic (ln - ln) transformation of both variables. The characteristics of the original (non-transformed) regressions are presented in Table 19. The group of fishes in the lnED/lnTL graph, which stay outside the main regression line is indicated by a box and shown separate in magnification (ED/TL). For abbreviations see Table 18.



**Figure 41.** Development of somatic indices (GSI, MLI, ILF, LSI, SSI, K) with growth (total length: TL in mm) in juvenile and adult common dentex (*Dentex dentex*). For abbreviation see Table 18.

## 4.8 Observations of abnormal developments

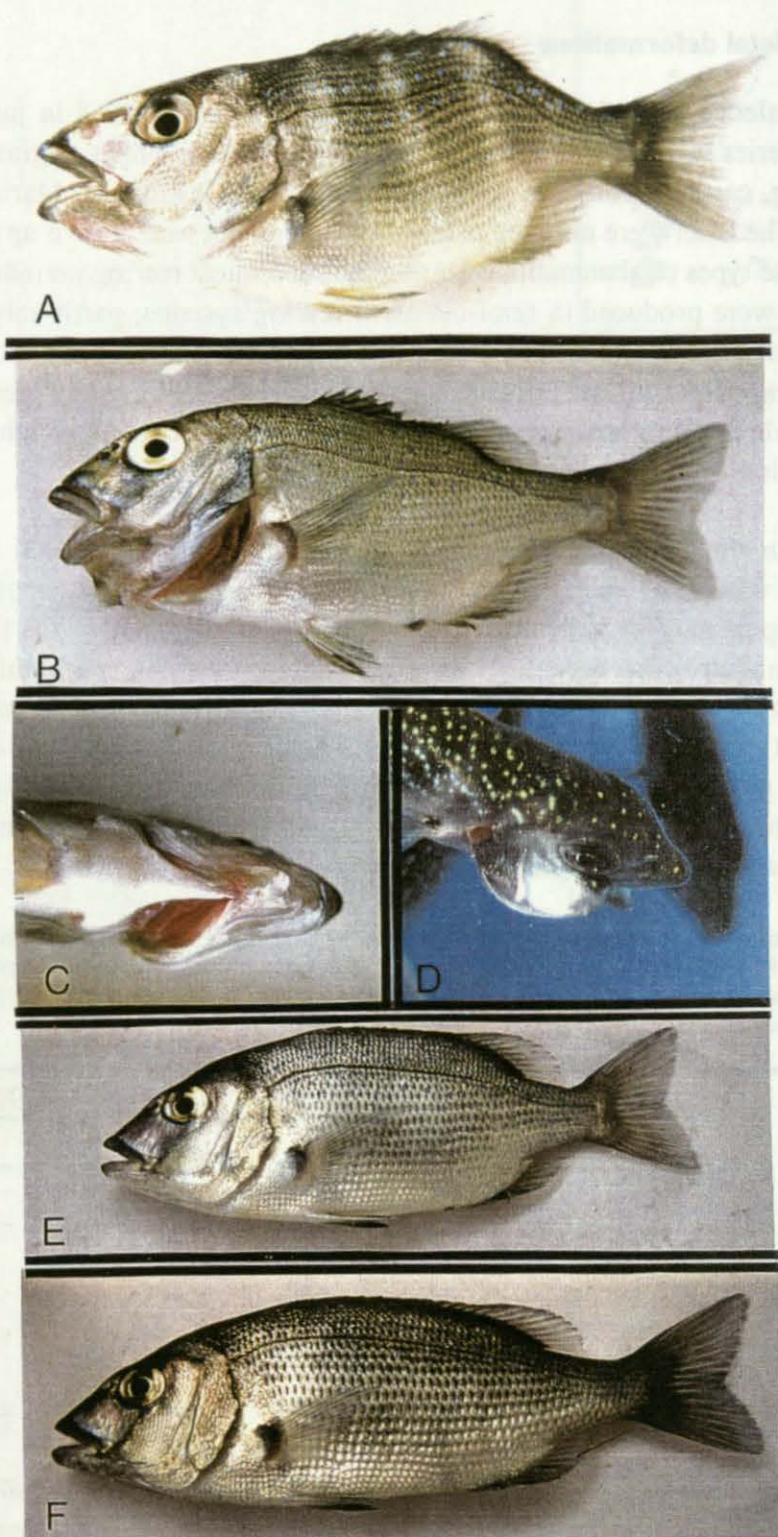
### 4.8.1 Skeletal deformations

A high prevalence of various skeletal deformations was observed in juveniles of the first production series in 1992 (EP 1 & 2) and in a batch of fish which originated from intensive larval rearing, carried out by the production team of the Institute of Marine Science in 1993 (Table 20). The latter were not used in any experiment, but were grown up to a size of 20-50 g to describe the types of abnormalities. During the subsequent rearing periods in 1993 and 1994, when larvae were produced in semi-intensive rearing systems, partly relying on the natural food chain of large seawater enclosures (mesocosms), abnormalities were less severe and occurred at moderate to low prevalences (Table 20). Beside skeletal deformations also an abnormal scale development during the 1994 rearing was recorded, which is described in the last part of this chapter.

All fish from the 1992 and 1993a rearing trials lacked a functional swimbladder. The swimbladder was easily visible on radiographs as a voluminous dark cavity located between the chordal spine and the body cavity. The unbalanced buoyancy of the fish due to the non-inflated swimbladder has been associated with a lordosis of the vertebral column in several studies (Kitejima et al., 1981, Daoulas, 1991). In fish of the extensive larval rearing productions (1993 b, 1994) a low to moderate number of fish with lordosis were detected, which could possibly be related to the missing inflation of their swimbladder. The positive relationship between the prevalence of lordosis and fish damaged by agonistic behaviour has been already shown during experimental period 8 ( $\Rightarrow$  4.5, Figure 32).

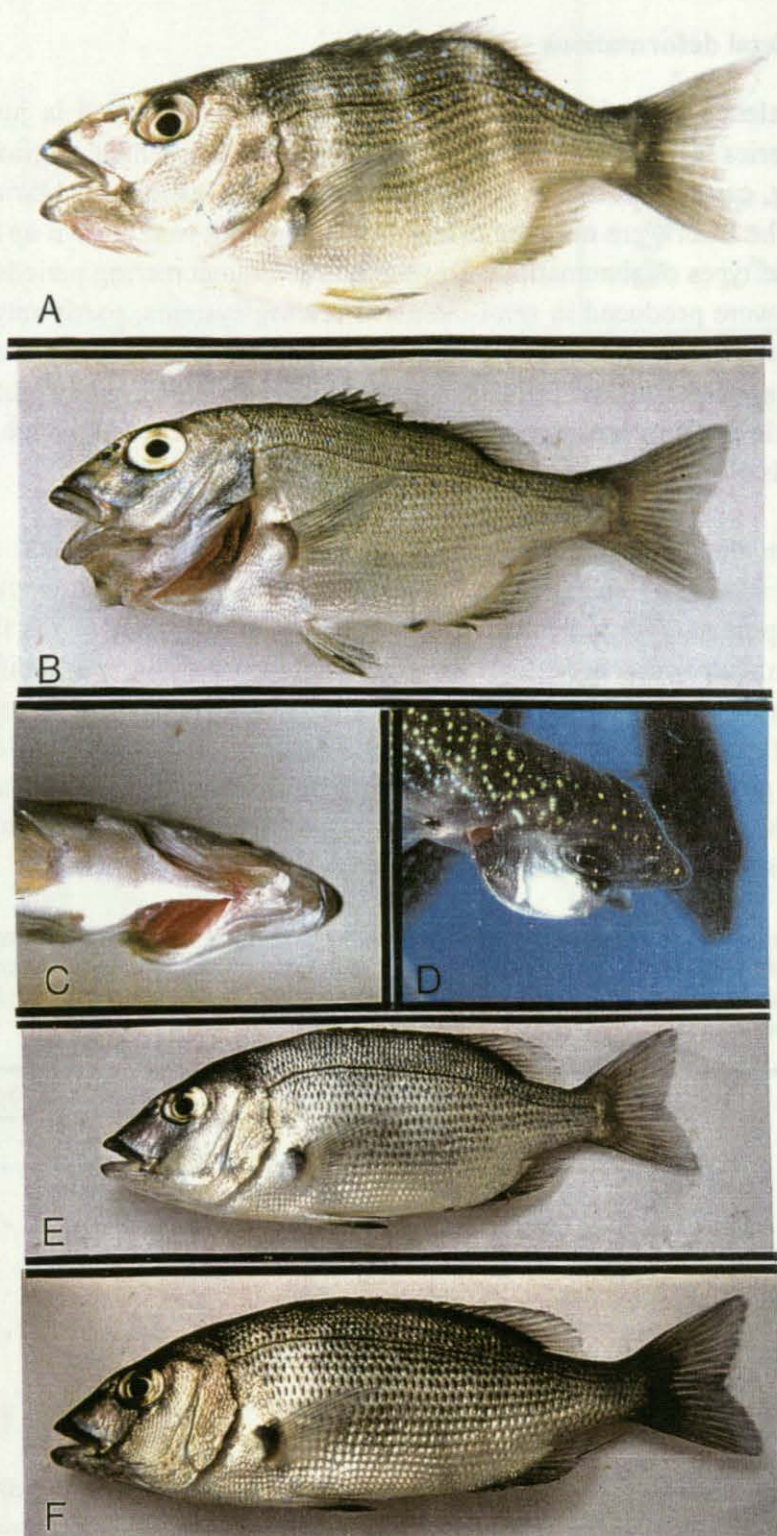
**Table 20:** Prevalence of skeletal deformations among juvenile dentex of four different production series. Examined fish in 1992, n=45 and in 1994, n=360; \* = not determined; -- = not observed. Percentage values bases on systematic counts of affected individuals of a population. Low, medium, high are estimates made by the researcher.

		1992	1993 a	1993b	1994
1	Twisted operculum	48.9 %	low	—	—
2	Protrusion of lower jaw	--	high	--	—
3	Crossbite	*	low	low	-
4	Fusion of vertebrae	4.44 %	high	*	*
5	Deformed ribs	42.2 %	*	*	*
6	Lordosis	44.4%	high	low	5.67% (low)
7	Scoliosis	low	low	low	—



**Figure 42.** Abnormal developments in juvenile common dentex (*Dentex dentex*) monitored during 4 production series. (A) strong lordosis, (B) 'short fish' displaying moderate lordosis and the twisted operculum syndrome, (C) Twisted operculum (ventral view), (D) Twisted operculum, dorsal view, (E) 'short fish' (F) normal developed juvenile.





**Figure 42.** Abnormal developments in juvenile common dentex (*Dentex dentex*) monitored during 4 production series. (A) strong lordosis, (B) 'short fish' displaying moderate lordosis and the twisted operculum syndrome, (C) Twisted operculum (ventral view), (D) Twisted operculum, dorsal view, (E) 'short fish' (F) normal developed juvenile.

The skeletal abnormalities which occurred during all experimental periods of this study were classified into 7 groups as following:

- (1) hypertrophy of the branchiostegal rays, also known as twisted operculum (Daoulas, et al., 1991) or swollen operculum (Barahona-Fernandes, 1982) (Figure 42 b-d, 43c)
- (2) protrusion of the lower jaw (42 b,c)
- (3) lateral shift of the inferior jaw bones, commonly known as 'crossbite'
- (4) fusion or compression of vertebrae (brachispondylose) (Figure 43 d). The fish body appears short in comparison to head size and body height (Figure 42 e).
- (5) curvature or reduction of ribs (Figure 43 e)
- (6) dorso-ventral curvature of the vertebral column 'Lordosis' (Figure 42 a)
- (7) lateral curvature of the vertebral column, 'Scoliosis'

(1) A hypertrophy of the branchiostegal rays occurred on either one or both sides of the head at a relative high frequency during the first production series (EP 1+2), when almost every second fish expressed this anomaly and at a rather low prevalence among the strongly deformed batch, reared under intensive larval conditions (1993a). During the following experimental periods (1993b and 1994) such a deformation was not recorded again (Table 20).

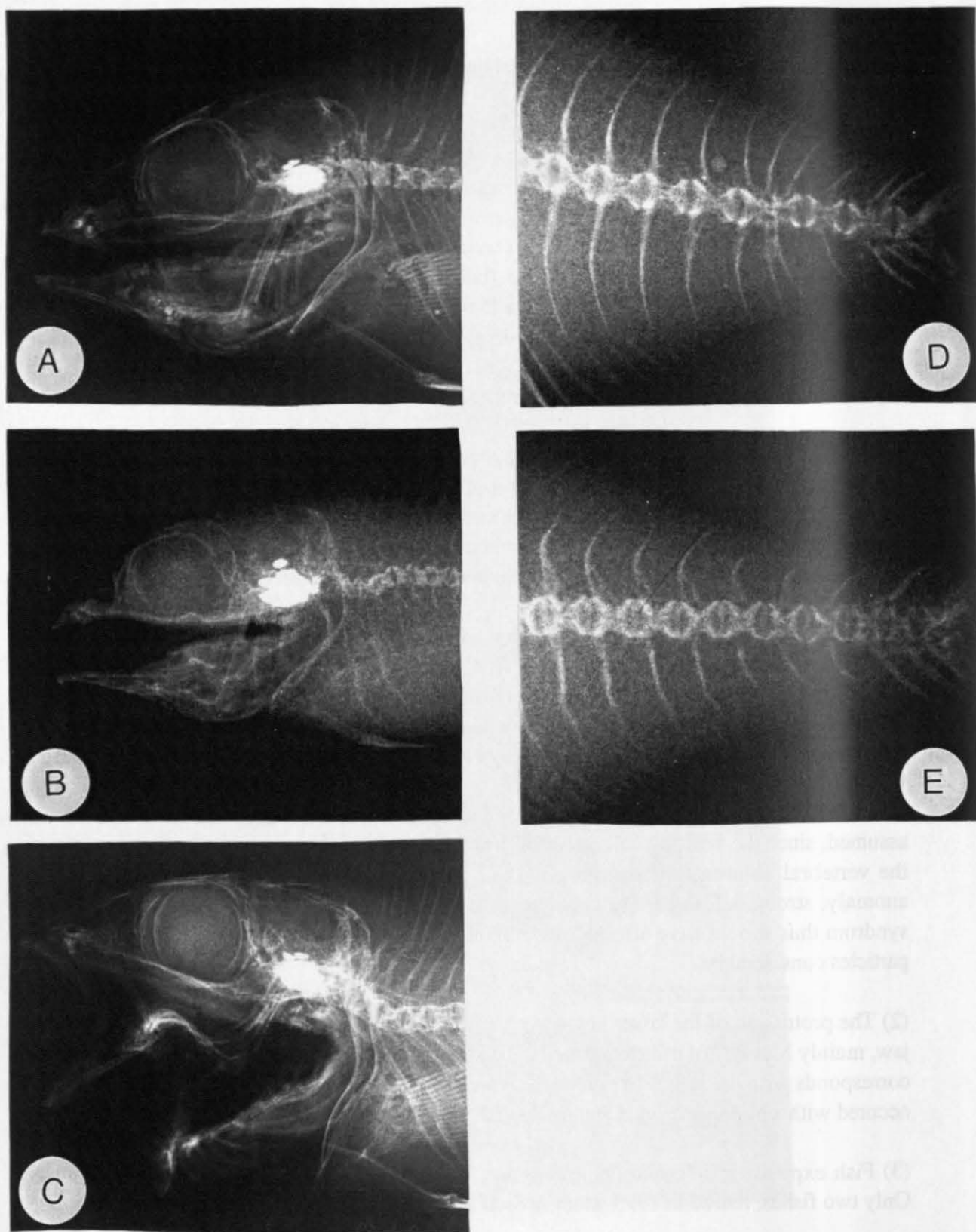
The radii branchiostegi (branchiostegal rays) are dermal bones, formed in a fold of skin, the branchiostegal membrane, which extends from the hyoid in the form of a caudally-directed flap thus covering the gills (Harder, 1975). Figure 42 b-d shows the laterally lifted operculum in juveniles (EP 1 & 2). The radiograph of a heavily affected fish head is shown in figure 43 c. The hypertrophy of the branchiostegal rays becomes obvious by the considerably enlarged ventral part of the mouth. Frequently, this anomaly was combined with a dorsal bend of the vertebral column just behind the head. A causal relationship between these anomalies was assumed, since the ventrally enlarged mouth area possibly caused a dorsally directed pressure on the vertebral column thus resulting in their sharp upward bend. As a consequence of this anomaly, strong deformed fish could not close neither their mouth nor their gill cavity. This syndrome thus should have affected respiration efficiency and the uptake and ingestion of feed particles considerably.

(2) The protrusion of the lower jaw was characterized by the hypertrophy of parts of the lower jaw, mainly Meckel's cartilage seemed to be affected, which is located between the dentale and corresponds with the posterior part of the lower jaw the articulare (Harder, 1975). This anomaly occurred with a high prevalence among the fish of batch 1993a (intensive larval rearing).

(3) Fish expressing a "crossbite" have been found rarely among the juveniles of this study. Only two fishes, reared in 1993 under favorable conditions, expressed this mouth anomaly.

(4) The fusion or compression of vertebrae (brachispondylose) (Figure 43 d) did occur in the caudal part of the vertebral column between the 19. and the 24. vertebrae. Due to the strongly compressed body of the vertebrae, four instead of two ribs insist at a common point on the vertebral column. In normal developed cultured fish and wild fish a total of 23-24 vertebrae





**Figure 43.** Radiographs of skeletal anomalies monitored in common dentex of the 1992 production (EP 1+2). (A) normal developed head, (B) ventral bend of the vertebral column near the head (C) hypertrophy of branchiostegal rays combined with dorsal bended vertebral column, (D) fusion or compression of vertebrae (brachiospondylose) (E) deformed neural and haemal spines in the caudal region.

were counted, which is characteristic for higher teleosts (Fiedler, 1991). Compared to the prevalence of other kinds of anomalies determined among fish of the first production series (EP 1+2), a relative low percentage 4.44 % of the examined fish exhibited this anomaly (Table 20). During the subsequent years, defined samples of fish had been photographed in weekly intervals during the 1993 experiment (EP 5) to monitor the morphological development. On these photographs frequently fish, which appeared relatively "short" compared to their body height were identified, which possibly developed also this anomal fusion of vertebrae (Figure 42 e)

(5) The curvature and reduction of the the neural and haemal processes (ribs) of the vertebrae was detected on the radiographs from fish of the first production in 1992 (EP 1+2) (Figure 43e). This type of deformation occurred at a high prevalence of 42.2 % among the examined specimens of this production. Since this kind of anomaly cannot be seen externally, no estimation for the subsequent years can be given.

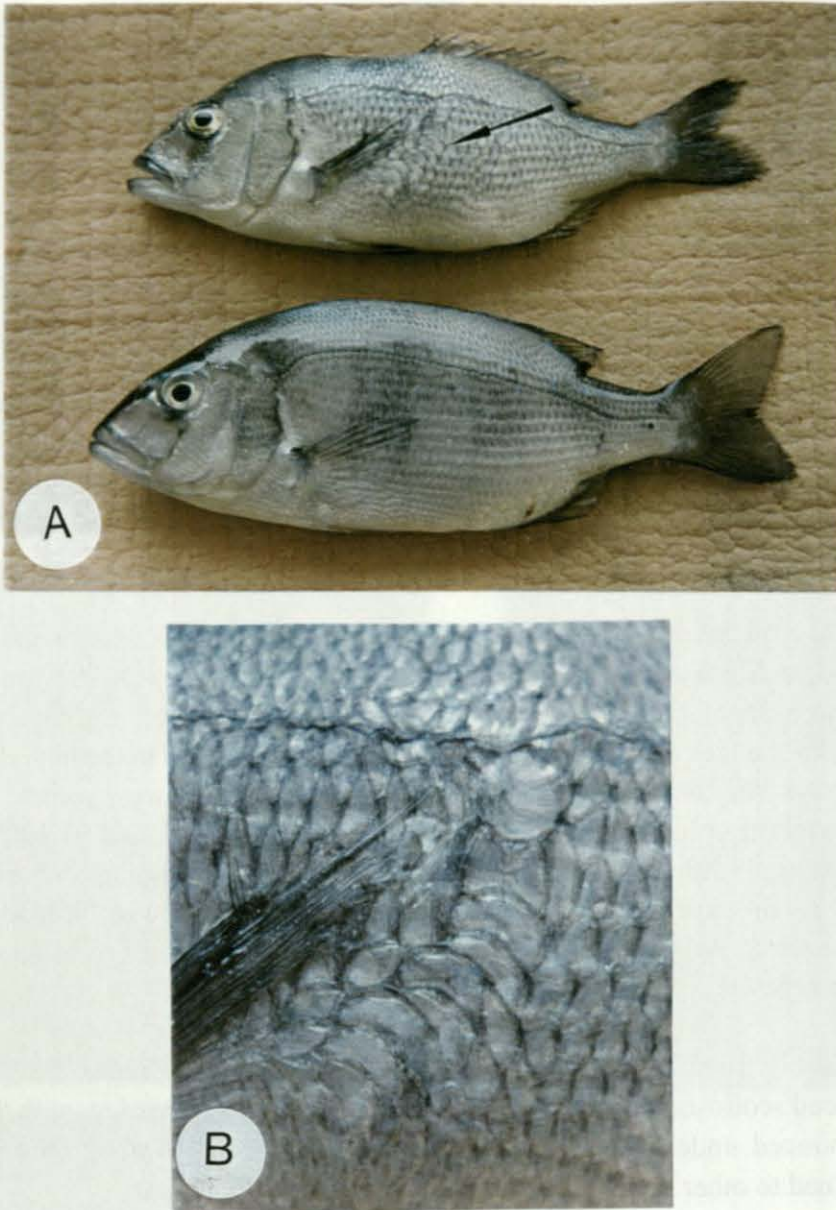
(6) Lordosis was expressed in 1992 in the most cases as a dorsally oriented bend of a relative short part of the vertebral column just behind the head (Figure 43 b+c). The vertebrae in this part seemed also to be compressed. This type of lordosis is different to the commonly known lordosis related to the lack of swimbladder and can possibly related to the hypertrophy of the branchiostegal rays (see anomaly 1). In 1993a a strong curvature of larger parts of of almost all parts of the vertebral column was determined. This batch contained to 100% deformed specimens, an example fish is shown in figure 42 a. During the remaining production series in 1993 and 1994 (main experimental periods), a moderate prevalence of lordosis most likely related to an uninflated swimbladder was recorded. These fish expressed an increased mortality due to agonistic behaviour (Figure 32).

(7) Scoliosis was observed seldom in fish reared under favorable conditions. Only 2 specimens with a pronounced scoliosis were found during the entire 3 years of rearing, with the exception of the fish produced under intensive larval rearing conditions. Among these, the rate of scoliosis combined to other skeletal deformations, was markedly higher.

#### 4.8.2 Abnormal scale development

At the end of the experiment on the application of immunostimulants in juveniles (EP. 8), a scale anomaly was observed for the first time in several fish. Abnormal enlarged scales were found in one to two dorso - ventrally oriented lines, below the lateral line organ (Figure 44 a+b). This fish also exhibited an abnormal short dorsal fin, with only 7 hard and 7-8 soft rays (normally/11), which inserted, instead near the base of the pelvic fins at the end of the pelvics, in line with the abnormal scales. Irregularities in form and positioning of scales could also be found above (dorsal) of the lateral line, but to a less severe extent.

The late detection of this scale anomaly, in fish already 5 months old of 30-45 g mean weight, is astonishing. Although figure 44 shows one of the most severe cases of this aberration and the observation of swimming fish from the top of the tank did not allow an easy detection of



**Figure 44.** Abnormally enlarged scales in common dentex (*Dentex dentex*). (A) Comparison of fish with abnormal enlarged scales (top) and a normal developed fish (bottom) (B) Magnification of abnormal scales.

fish with scale anomalies, it would have been expected that during the weekly weighing of all fish such a strong deformation had been detected. It can be therefore assumed that either the expression of this scale anomaly started relative late in ongrowing juveniles, or that the prevalence was so low that these fish have been overseen during the routine weighing, when fish were not individually measured, but weighed in groups of 20-30.



## 5 DISCUSSION

*Dentex dentex* is considered to be an excellent aquaculture candidate, of its reproductive biology and growth performance. The objectives of the present study aimed on a first determination of several biological key parameter, relevant for the cultivation of the common dentex (*Dentex dentex*) with regard to aquaculture purposes. Among the huge variety of about 30.000 fish species, only about 100 species are presently cultivated (Pillay, 1992). These species do manifest several biological traits in common, essential for the profitable mass production of fish. The most important criteria aquaculture species must fulfill are: (a) the production of high numbers of gametes under controlled conditions (Marino et al., 1995; Faranda et al., 1985), (b) the ability to adapt to captive conditions and handling stress (Klinger et al., 1983; Leatherland, 1993; Salonijs and Iwama, 1993), (c) the acceptance of commercially available feed products (Bromley and Howell, 1983; Carlstein, 1993; Watanabe & Sakamoto, 1992; Tandler et al., 1982; Jobling, 1986), (d) high survival rates, especially during pre- and on-growing periods (Kvenseth & Øiestad, 1984; Degani & Levanon, 1983) (e) a high growth potential during all life stages until marketable size is reached (Hogendoorn, 1983; Jones, 1984; Divanach et al., 1993), (f) the efficient conversion of feed-protein and feed-energy (Torres-Pereira, 1990; Soofiani & Hawkins, 1982; De la Higuera et al., 1989) and (g) a low disease susceptibility under culture conditions (Snieszko, 1974; Paperna, 1984; Gray et al., 1985).

Besides these biological performance characteristics, economic considerations are as important or even more important factors within the selection process of suitable species for culture (Chua & Teng, 1980; Jones, 1984; Pillay, 1992). Although a market analysis was not part of the present study, the high wholesale price, as well as the excellent consumer acceptance of the common dentex throughout the entire Mediterranean area were important factors when selecting the species for this investigation.

In the present study, juvenile and on-growing common dentex were studied in several rearing experiments during a 3 years period to determine the range of biological traits, relevant for aquaculture. The results concerning growth performance, survival rates, and feed conversion efficiencies will be compared with the performance of other aquaculture species and discussed in regard to their possible effects on commercial production. The relationship between social interactions, manifested commonly in agonistic behaviour or cannibalism, and their regulating factors, such as population structure, food availability and water current speed influence species performance in critical ways, in particular food uptake, growth, and survival rate (Brownell, 1989; Knights, B., 1987; Kaiser et al., 1995; Folkvord & Otterå, 1993). Therefore, the social performance of the common dentex under culture conditions was validated in regard to the culture technique employed and adapted to the biological traits of this species. The repeated outbreak of infectious diseases, observed during the whole series of experiments, are considered in light of an inhibited immuno-competence, which was induced by repeated handling stress. Finally, the possibilities to protect a "new" species, such as the common dentex against infectious diseases caused by bacteria or parasites is discussed in view of the application of immunostimulants.

## 5.1 Food acceptance

The feeding behaviour of a species determines the feeding strategy to be employed under culture conditions as well as the type of food which can be used to achieve optimal food utilization. During the different rearing periods, juveniles and ongrowing fish accepted a variety of fresh, wet, moist, and pelleted diets fed either by hand or by an automatic feeder (dry diets). Today, most intensive aquaculture systems mainly rely on complete dry pellets which offer important advantages to fresh and moist diets, such as a defined constant composition, good storage and feeding properties as well as a high water stability. Although fresh and moist diets have been widely used to start the culture of "new species" (Moree et al., 1996; Iglesias et al., 1987; Howell, B.R., 1984), during the weaning period from life to artificial diets (Cadena Roa et al., 1982; Métailler, 1990) and in the production of fish, which do not easily accept dry pellets such as the *Seriola*, *Seriola quinqueradiata* (Watanabe, T.; Sakamoto, H, 1992; Tachibana et al., 1992), there is increasing concern about the high water pollution potential when using such low stability diets.

Juveniles of about 1-2 g mean weight could be successfully adapted to feed on dry pellets, which were composed to fit the nutritional requirements of sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), after a prolonged weaning period including different composed moist pellets as transitory feeds. However, the performance of culture, when juveniles and ongrowing fish fed exclusively such dry pellets, was low compared to fish fed on a self-prepared moist pellet. This low performance was expressed by considerably reduced growth rates, increased mortality rates mainly due to agonistic behaviour as well as an increased disease susceptibility.

Therefore, this study suggests, that the common dentex is able to accept dry diets from the behavioural point of view, but shows a poor production efficiency when compared with groups fed on moist diets. The possible reasons for the difference of these two types of food for dentex are further discussed in chapter 5.4.

The daily activity cycle has been shown in several fish species to be influenced by the interaction of biological timing and abiotic parameters such as light-quality and -quantity and tidal phases (Eriksson, 1978; Manteifel et al., 1978; Champalbert, 1989). In addition, it has been demonstrated in fish that feeding considerably entrains circadian activity rhythms. Fluctuating blood cortisol levels influenced by feeding frequency and feeding time explain this change of locomotory activity (Spieler, 1992).

Juvenile dentex (2-3 months in age, EP 2) expressed the highest food consumption at the evening feeding (4 feedings a day). Contrary to this trend, ongrowing fish of about 1 year in age showed increased appetite during the first feeding of the day, irrespective of feeding frequency (2-3 times a day) or the day time of the first feeding. Since these determination were collected during relative short time periods and appetite was fluctuating considerably, no clear daily feeding activity rhythm in juvenile and ongrowing dentex was obvious during the conducted experiments. As it was assumed that the common dentex is a visually oriented predator, no feeding trials during the night were carried out.

## 5.2 Effects of agonistic behaviour on culture efficiency

Agonistic behaviour is a common phenomenon in many fish species, especially expressed during larval and juvenile life stages (Smith and Reay, 1991; Dowd & Clarke, 1989). Such behaviour can be expressed as threatening (Knights, 1987), nipping (biting) of conspecifics (Brownell, 1989), or as true cannibalism (Bry et al., 1992, van Damme et al., 1989), when parts or the entire conspecific is ingested by the cannibal. During this study, no true cannibalism between developed juveniles ( $> 1\text{g}$ ) and ongrowing fish took place, however, agonistic behaviour was strongly expressed. The biting behaviour between tank mates was found to cause the largest part ( $>50\%$ ) of the mortality rate among healthy juveniles of about 2-40 g mean weight in this study (Table 14, Figure 19). During the experimental period in 1993, the intensity of agonistic behaviour was related to the type of feed administered. Fish fed on moist diets expressed significantly reduced mortalities due to biting behaviour and the relative size of predated specimens was considerably reduced within this feeding regime compared to fish fed on commercial dry pellets. Such diet related effects concerning the intensity of agonistic behaviour have been already shown for several fish species (Bromley and Sykes, 1985; Brownell, 1989; Holbrook & Schmitt, 1992; Kaiser et al., 1995). Smith and Reay (1991), who reviewed the literature of cannibalism in teleosts, found a negative relationship between cannibalism and feeding level in 62 fish species, including both aspects, food quantity as well as food quality.

In several studies on cannibalism, the influence of size differences between predator and prey was used to establish "safe" limits of size ranges within which cannibalism can be determined under culture conditions. The present study tried to estimate this range by using weight difference between prey and predator. Since the predator (aggressor) could not be identified individually during this study, the mean weight of prey was compared to the mean weight of the respective tank population (range of mean weights: 2.4-31.3 g). The size of prey was found to be considerably smaller in the moist pellet fish (34.5% of tank mean weight) compared to juveniles reared on dry pellets (53.8% of tank mean weight). Predators therefore were  $2.9 \times \text{TW}$  ( $1.4 \times \text{TL}$ ) and  $1.9 \times \text{TW}$  ( $1.2 \times \text{TL}$ ) the size of prey for the moist and dry diets respectively. Although we do not know which fish of a tank population were the main aggressors, it is well known from other species that commonly the largest  $\alpha$  – animals are the main predators. Therefore it is realistic to predict a scenario with cannibals belonging only to the largest specimens ( $\approx 200\%$  mean weight  $\Rightarrow$  Figure 15). In this case the predator-prey size relationship would have been  $5.8 \times \text{TW}$  ( $1.76 \times \text{TL}$ ) and  $3.7 \times \text{TW}$  ( $1.53 \times \text{TL}$ ) for the two test diets. However, this relationship is probably not fixed for a species in culture, but probably varies within a range characteristic for each life stage and depending on rearing conditions such as environment (refugees), current velocity, stocking density or nutrition (Rosenthal, 1970; Appelbaum, 1980; Degani & Levanon, 1983; Kushnirov, & Degani, 1991; Hecht & Pienaar, 1991).

Similar prey - predator size ratios have been identified in other species, such as highly cannibalistic eels of fingerling size (Knights, 1987). Agonistic behaviour was most marked when the larger eels were at least 1.5 times the weight of the smaller. In juvenile cod, a minimum relationship between predator standard length and prey standard length was found to

be 1.3-1.5 in fish of 22.5 mm SL, assuming that a predator can catch fish with a body height less or equal to its mouth gape (Otterå and Folkvord, 1993), and Parazo et al. (1991), who investigated size dependent cannibalism in hatchery-bred seabass (*Lates calcarifer*) found that predators possessed about 1.49-1.64 times the total length of their prey.

Considering own results and experiences from other species, the size range of juvenile dentex should not exceed  $\approx 1.5 \times TL$  ( $\approx 3.5 \times TW$ ), in order to reduce the severe effects of agonistic behaviour under culture conditions.

The Coefficient of Variation, the standard deviation expressed as a percentage of mean weight, is a commonly used measure to describe variability of size in fish. This measure has also been employed to evaluate size-dependent cannibalism (Folkvord & Otterå, 1993 - *Gadus morhua*; Kushnirov & Degani, 1991 - *Anguilla anguilla*; Appelbaum, 1980 - *Anguilla anguilla*). The CV values determined during the experiment on the effects of dry and moist diets (EP 5) were significantly elevated in the moist diet group compared to the tank populations fed with dry pellets. Agonistic behaviour, however, was not related to the CV as expected from other studies, but significantly reduced in the moist pellet group which expressed the higher coefficients of variation. The type of diet administered seemed to influence agonistic behaviour to a stronger degree than relative size variability.

However, the suitability of the CV to describe size variation of fish populations with regard to cannibalistic potential is limited to near to normal size distributions. In growing fish populations, however, a skewed size distribution is more typical (Degani et al., 1988; Houvenaghel & Huet, 1989; Folkvord & Otterå, 1993; Efthimiou, 1992). Therefore, different size distributions between the two test groups could also explain the inconsistencies observed between the CV and the intensity of agonistic behaviour during this study.

The analysis of size distributions (Figure 15) revealed a significantly positively skewed size distribution in the moist diet groups at the start of the experiment. However, during the subsequent weeks, this effect progressively diminished and skewness reached lowest values during the last week of the experiment, when the relative size difference between smallest and largest fishes was especially pronounced ( $> 10 \times$  total weight). This rather untypical development was probably caused by the high mortalities among the smallest individuals, which had been also found during this experiment (Figure 20). Especially from experiences with the culture of young eels, an increase of positive skewness is known (Houvenaghel & Huet, 1989).

It becomes obvious that not one single measure can be standardly used to evaluate the cannibalistic potential within a fish population under culture conditions. Furthermore, a detailed characterization of size distribution, with regard to size variability (coefficient of variation), size range and the shape of the size distribution (skewness, kurtosis) is necessary to take into account the above discussed interactions with agonistic behaviour.

Knights (1987) demonstrated that larger eels attack more readily, are more often dominant in threat encounters, are less active, consume more food, and show higher growth rates and lower



mortality compared to smaller, subordinate eels. In the present study, agonistic behaviour was quantified in terms of biting attacks leading to the death of the attacked individual. This specification of agonistic behaviour does not include the social stress effects on the inferior fish. Such social interactions, however, could have influenced growth performance of smaller fish considerably, especially in rearing experiments with pronounced cannibalism. In subordinate eels, a number of parameters, mainly characteristics of the General Adaptation Syndrome of Seleye's stress principle (Seleye, 1956), were significantly different to dominant specimens. In subordinate fish the total leucocyte counts were lower, the plasma cortisol concentration elevated, but in some subordinate fish it also decreased. Decrease in liver glycogen and an increase in blood glucose and lactate were also determined (Peters et al., 1980). This indicates that the establishment of a hierarchy can cause considerable stress in fish of a tank population (Mazeaud and Mazeaud, 1981; MacArthur et al., 1984; Pickering, 1993; Salonijs and Iwama, 1993). Furthermore, under aquaculture conditions fish cannot escape from social stress, which has to be considered therefore as a chronic stress, leading to elevated blood cortisol levels over prolonged periods (Schreck, 1981). Under stress, metabolism is switched from an anabolic state, in which energy is taken up and stored, to a catabolic state, in which energy reserves are broken down (Pickering, 1993). Therefore, even the mere presence of dominant individuals may cause loss of appetite and/or low food conversion by subdominants. Thus, the significantly reduced growth rates and food conversion efficiency determined in fish fed on dry pellets, was possibly related to the elevated level of social stress within these feed groups compared to the test groups fed on moist diets.

The high mortality rate occurring during the 1993 (EP 5) rearing experiment due to agonistic behaviour was considerably reduced in 1994 (EP 8), when rearing conditions were changed by means commonly known to reduce cannibalism in teleost fish. These included (a) the size grading of the fish into two size classes at the beginning of the experiment, (b) the elevation of the mean stocking density (1.94 vs 7.75 kg/m<sup>3</sup>) and (c) a considerable increase of water current speed. These modifications led to an overall reduction of the mortality related to agonistic behaviour from 24.2% in 1993 to 3-5% in 1994 (for a similar fish size and a similar moist diet). The effect of high stocking densities on agonistic behaviour is especially expressed in species which establish and defend territories within their confinements. In its natural environment the common dentex is known as a solitary, strictly predatory fish, - typical attributes for territorial species. In intensive stocking densities, the available space per fish is small, thus inhibiting the formation of territories. The number of aggressive encounters or biting often decreases under such conditions.

High current speeds force fish to swim actively against the current. Attacks, however, often require a change in swimming direction, which is energetically more expensive for fish. The increased drag acting on fishes leaving the optimal streamline position, e.g. to attack conspecifics, results in a back-drift of the aggressor, making biting attacks and threatening encounters less attractive and less secure. This results often failure to hit the prey while also discouraging aggressive individuals to continue their aggressive behaviour (Rosenthal, 1970).

In larger fish (11-15 months, 100-300 g), the biting behaviour was still present, but at a considerable reduced intensity. Fish with wounded tails were frequently observed, but only few



individuals died due to these damages. However, even low mortality rates of these large fish cause considerable economic loss to the farmer, and individuals with damaged tail fins can usually not be sold at regular price, since the outer appearance is an important factor for the market value of high priced fish. Rearing conditions during this period could not be arranged in order to establish an environment shown to reduce aggression in juveniles. Especially the current velocity was low, compared to the swimming ability of the fish. Therefore, a modification of rearing conditions with regard to factors which positively influence agonistic behaviour can be assumed to reduce aggression in ongrowing fish when compared to the findings of the present study.

The rearing experiments with common dentex discussed above indicate (a) the high potential for agonistic behaviour in juveniles (1-40 g) and its persistence in larger fish (100-300 g) under intensive culture conditions, (b) the possibility to reduce agonistic behaviour to a relatively low level by measures such as a well balanced nutritional regime, grading, stocking density and a relatively high current speed (c). The present study suggests, that agonistic behaviour can be reduced most effectively by increasing the current speed to a level that stimulates rheotaxis in common dentex.

The change of aggressive behaviour in fish in course of domestication, - "the process of adaptation of organisms to an environment provided by man" (Ruzzante, 1994) - is an important, but often overlooked factor during the introduction of new species. However, the question whether domestication increases or decreases agonistic behaviour is still a matter of dispute (Ruzzante 1991 vs. Swain, 1991), since criteria for determining such effects have not been clearly defined yet.

Heavy cannibalism among postlarvae and early juveniles and agonistic behaviour in older specimens continuing still in ongrowing fish, as reported from several studies including the present investigation for the common dentex, is a limiting factor for the commercial production of a species. However, high mortalities due to cannibalism have been reported also from presently successful aquaculture species such as the seabass, up to 97% mortality (Katavic et al., 1989;) and up to 42 % in the European eel (Degani & Levanon, 1983). Intensive cannibalism is often observed during the start-up phase of culture, when rearing technology is still not well adapted to the requirements of the species and suitable diets have not been developed. The success of modifications in the rearing technology described in the present study, suggests that similar improvements are possible in the aquaculture of *Dentex dentex*.

### 5.3 Growth performance

#### Growth potential

Growth performance is of crucial importance to the aquaculture industry with implications on production costs. The expression of growth potential under culture conditions, the actual growth rate, strongly depends on a variety of factors of which the most important are: the genetic growth characteristics of the strain or stock of a species used, nutritional requirements (quantity and quality), behavioural aspects in relation to stocking density and social hierarchies, water quality (including temperature), disease susceptibility and responses to external stressors

(crowding, handling etc.) (Brett, 1971; Hephher, 1988; Weatherley & Gill, 1987; Hogendoorn et al., 1983; Kawamoto, 1961; Pickering, 1993; Francescon et al., 1988; Higgins & Talbot, 1985). Adams and McLean (1985) described well the complex, integrative character of external factors affecting growth with their statement:

*"Growth is the ultimate expression of fish health or condition because it integrates all the biotic and abiotic variables acting on an organism and reflects secondary impacts of chronic stress".*

The evaluation of growth rates with regard to the expected growth performance of a new species under intensive culture conditions requires therefore a thorough discussion of the most important aspects.

The experiments of the present study focused mainly on juvenile fish (2-5 months after hatching) within a size range of about 2-40 g, but also fish aging about one year reaching marketable size at the end of the second summer were studied. Juveniles reached a mean weight of 2 g about 65-75 days after hatching, at relatively high temperatures of 22 - 26 °C. This indicates a high growth potential already during larval and post larval stages since other warm-water species such as the European seabass (*Dicentrarchus labrax*), which is reported to reach 1.5-1.7 g in 90 days (Person Le Ruyet et al., 1991) and the gilthead seabream (*Sparus aurata*) reaching about 1 g in 90-100 days (Berg & Cittolin, 1987) express slower growth. However, the low survival rates of 2-5% obtained during these rearing periods do not allow to generalize these results. Massive cannibalism starts very early among dentex fry (25 days after hatching, 14 mm total length in Franicevic, 1991) and may lead to selective survival of fast growing individuals, resulting in an overestimation of growth rates. Increasing survival rates, going ahead with optimized larval rearing techniques will possibly result in lower growth rates on the population level. The development of larval and postlarval life stages of the common dentex in relation to nutrition and rearing techniques have been intensively studied parallel to the present investigation by Koumoundouros (1996).

Highest growth performance and best food conversion efficiency were achieved with moist diets in this study. These values were chosen therefore for the comparison between common dentex and other aquaculture species (Table 22). From the listed publications used for comparison in Table 22, always the highest growth rate of different treatments (e.g. for different feeds) has been selected.

The growth rates determined for juveniles during this study compare favorably with high values reported for other warm-water species of similar size, reared at similar temperatures (Table 22). Other sparids used in aquaculture of the Mediterranean area, such as the gilthead sea bream (*Sparus aurata*) are reported to express lower growth rates (Kissil and Koven, 1987; Tandler et al., 1982), than the common dentex.

Growth of juveniles during the first year of rearing (experimental periods 1 + 2) was reduced compared with the subsequent years (1993 + 1994). This lower growth rates are likely related to the poor weaning regime applied during this year and the severe skeletal deformation observed in these fish. A relationship between the immediate change from live food to dry weaning diets and the occurrence of skeletal deformations was assumed. During the subsequent

**Table 22:** Favourable Specific Growth Rates (SGR % bw \* day<sup>1</sup>) of warm-water aquaculture fish, including several species already successfully cultured in the Mediterranean area, compiled from literature and the present study.

Species	SGR	Fish size	Water temp. °C	Reference
<i>Ictalurus punctatus</i>	5 %	3-12.5 g	28	Stickney et al., 1972
<i>Clarias lazera</i>	6-7 %	0.3-40 g	25	Hogendoorn, 1983
<i>Sparus aurata</i>	2.1- 3.3 %	1.3-5.3 g	25-28	Kissil and Koven, 1987
<i>Sparus aurata</i>	1 %	10 g	25.6	Tandler et al., 1982
<i>Sparus aurata</i>	1.5 %	1-12.33 g	20	Kalogeropoulos et al., 1992
<i>Scophthalmus maximus</i>	2.5-2.9%	5-10 g	*ambient 14.4-17.8	Iglesias et al., 1987
<i>Mugil capito</i>	1.40%	2.50 - 9.76	23	Papaparaskeva-Papoutsoglo & Alexis, 1986
<i>Anguilla anguilla</i>	1.95-3.33 %	0.13-0.38	23±2	Wickins, 1987.
<i>Anguilla anguilla</i>	1.49 %	2-5 g	25	Jespersen, 1989
<i>Dentex dentex</i>	8.69 %	3g	25	present study
<i>Dentex dentex</i>	3.11%	30 g	25	present study

\*optimal temperature for growth 18-20 °C

years, juveniles were weaned with fresh and moist diets over an extended period (4-6 weeks) before fed with dry diets, which resulted in higher survival rates and a low prevalence of abnormal fishes.

The specific growth rate of fish is size dependent and decreases with increasing individual weight of fish. Brett (1971) and Ricker (1979) have provided early proof of this fact when studying salmonids. However, age and size is probably only one reason for the reduced specific growth rates that were determined during the rearing trial with ongrowing dentex (180-310 g mean weight, EP 6), when compared to the growth rates of juveniles in the size range of 2-40 g. Factors such as unfavourable rearing conditions (tank dimensions, stocking density), inadequate feeding frequency or nutritional deficiencies are suggested inhibiting growth during this rearing trial. Several arguments for this assumption are given below.

The relationship between the log SGR and the log mean weight determined over a range of fish sizes should generally result in a linear regression characterized by a slope in the range of -0.4 (Brett, 1971; Jobling, 1983). The low value (-0.589) from the present study suggests that the larger fish did not fully exhaust their growth potential.

The relative low increase of growth rate from about 0.4 % to 0.6% SGR during the 13 weeks lasting experimental period, when ambient water temperature increased from 16.1 to 25°C at

the same time, do also not meet the expected temperature dependent increase of growth rate, known from other fish species. For example, a 5 °C increase of water temperature (15 to 20 °C) doubled the SGR from 0.46% to 0.97% in juvenile seabass (Hidalgo & Alliot, 1988) and also in sockeye salmon (*Oncorhynchus nerka*) an elevation from 5 to 10 °C almost doubled the maximum growth rate from about 0.7 % to 1.3 % (Brett et al., 1969). Thus, an increase of water temperature of 9 °C, within a range which is known as favourable for growth of many fish species of the Mediterranean regions (20-25 °C) should result in a 2 -3 fold of the specific growth rate. On the other side, the total size of the dentex increased from 181 to 280 g during this experimental period and as stated already above, this growth will result in a size depended decrease of maximum growth rate. This reduction of maximal growth rate, when calculated after the equation:  $\log \text{SGR} = 1.152 - 0.446 \log \text{mean weight}$  should be in the range of only 20 % and thus do only partly explain the reduced growth rates determined during experimental period 6 with ongrowing fish ( $\approx 50$  % the expected growth rate). During the same period, the daily food rations increased considerably with increasing temperature and almost threefold. This increase of maintenance requirements due to higher metabolic rates in fish, is well within the range for seabass and seabream (Alma, 1994). Considering the relationship between specific growth rate (SGR) and fish size and specific growth rate and temperature, discussed above, growth rates during the experimental period 6 did likely not express the full growth potential of common dentex during the second summer.

Although food ration seemed to increase normally with increasing temperature, a reduced feed intake at higher temperatures due to an inadequately set feeding frequency might have also contributed the observed reduced growth rates. During the entire experiment (EP 6) only two meals per day were applied, which was perhaps not sufficient for higher temperatures (Brett et al., 1969; Seymour, 1989; Kayano et al., 1993). Since gastric evacuation is strongly temperature dependent in cold-blooded animals, more frequent feeding could have resulted in higher growth rates. This suggestion is supported by the relatively high food conversion efficiency, observed during weeks 7-13, when compared to lower temperature periods (week 4-6). Best food conversion is usually gained at sub maximal rations and growth rates (Brett, 1971, Weatherley and Gill, 1987, Hephher, 1988).

However, although the here discussed size and temperature related effects on growth rate can be applied to many species, their transfer to the common dentex, an almost unknown species can only be of speculative nature. Therefore, the unexpectedly low growth rates determined in ongrowing fish may also be due to a reduced growth potential of the common dentex during the second year of its life cycle.

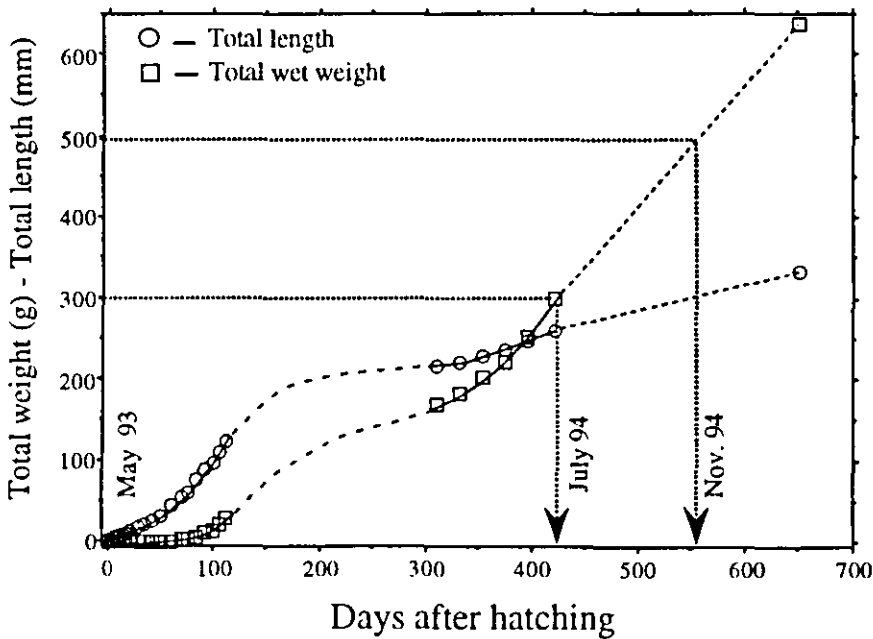
#### Implications for growth to marketable size

The time required to reach marketable size and the season when the major part of production can be sold are important criteria for fish farming management. In the Mediterranean region, the typical marketing weight of fish from aquaculture production concentrates on plate-sized fish of about 300-500 g. (Josupeit, 1995a; White, 1996). Figure 45 shows the length and weight increase of common dentex over a 22 months tank culture period (ad libitum feeding with moist pellets). Since growth was not determined during the late summer and winter months, a reasonable "guesstimate" was used for the first year and a linear interpolation during the second winter. These approximations are probably strongly biased because of the reduced

water temperature during winter. A more steep rise during late summer and autumn, when water temperature was still high in the Cretan Sea, and a more flattened curve during winter can be assumed as realistic when using ambient temperatures. The curves calculated here, especially during the second year are therefore conservative estimates for the determination of marketable size.

Marketable size was reached after 14-18 months after hatching (12-16 months from 1 g initial weight). This is well in the range of established aquaculture species such as the red sea bream (*Pagrus major*), reaching 600-700 g in 18 months (Foscarini, 1988), the gilthead seabream (*Sparus aurata*), reaching 200 g in 10 months and 390 g in 15 months from 1 g fry (Divanach et al., 1993), and turbot (*Scophthalmus maximus*) reaching a mean weight of 500 g in about 10 months from 9.7 g initial weight and fed with moist pellets (Iglesias et. al, 1987). The natural reproduction period from April to June and the high growth rates under favourable temperature conditions allow to produce fish that reach marketable size during summers, a time when supply from fisheries is commonly poor in the Mediterranean area and the wholesale price for marine fresh fish relatively high.

It may be assumed that even higher growth rates can be obtained when dentex are grown in net cages because of more favourable conditions, such as better water exchange and current speed, known to promote growth in salmonids (Jobling et al., 1993). The frequent handling stress during the experimental periods was additionally a growth inhibiting factor as shown during experimental period 8, when growth rates were significantly suppressed due to cumulative handling stress. However, due to size selective mortality and growth, an overestimation of growth potential should also be considered, especially when species with high mortalities related to agonistic behaviour are cultured (Oterå, 1992).



**Figure 45.** Growth curves of total length and wet weight gained from rearing trials with common dentex (*Dentex dentex*) under ambient conditions (Temp. : 13 - 26 °C) at the north coast of Crete (Greece). Dotted part of the curves are hand fitted (day 115-300) or linearly interpolated (day 420 - 600). The dotted lines (arrows) indicate the period when fish reach marketable size (300-500 g).

### Food conversion

A highly efficient food conversion is an important characteristic of aquaculture species. Generally, low food conversion efficiency results in high production costs and unacceptable environmental pollution. These effects are especially pronounced in species which are fed high protein, high energetic feeds, such as most carnivorous fish and crustaceans (Ackerfors & Enell, 1994; Talbot & Hole, 1994; Nijhof, 1994). Since food is the largest single cost factor for a fish farmer, and increasing demands of fish meal based feeds will lead to even further increasing feed prices (Ratafia, 1995), the importance of efficient food conversion will become even more stressed in the future. Environmental impact considerations have already led to regulations of production systems. Maximum values for food conversion factors is one measure, which is used in order to reduce negative environmental impacts of intensive aquaculture systems. This trend will continue in the next future and restrict the culture of species with low food conversion efficiencies (Rosenthal, 1994; Talbot & Hole, 1994).

Juvenile common dentex were able to efficiently convert high-protein high-energy pellets. In line with the high growth potential, highly efficient food conversion rates (food dry weight per unit biomass wet weight gain) with a mean of  $\approx 1$  were determined during the experimental period 5. However, the following year (EP 8) considerably elevated food conversion factors of 1.44 (mean) were determined for similar sized juveniles under modified culture conditions. Optimal food conversion rates reached with juvenile common dentex were similar to the conversion rates found by Hogendoorn (1983) for African catfish fingerlings (FC = 0.58). Mean conversion rates were comparable to other warm-water aquaculture species, e.g. the European eel (*Anguilla anguilla*) which reached an optimal value range of FC 1.3-1.8 (Koops, 1991; Seymour, 1989), and the gilthead seabream (*Sparus aurata*) which achieved optimal values of FC = 1.62 (Kissil and Koven 1987).

The less efficient food conversion obtained during the 1994 rearing period (EP 8) was related to markedly higher food consumption rates, indicating an increased energy requirement during this period. The most probable explanation for this difference can be found in the increased water current, which was applied during this rearing period, in order to reduce agonistic behaviour (see 5.3). Brett and Groves (1979) state within their review on energy requirements in fish that "It is locomotion that is metabolically costly for fish" compared to the rather low maintenance requirements of fish. Thus, even small changes in swimming speed induced by the increased water current could increase energy requirements considerably, and therefore resulting in the observed difference in food conversion efficiency. Other reasons, such as the increased cumulative handling stress, which was part of the experiment during the 1994 rearing or differences in stocking density and social stress may have also contributed to the elevated energy requirements (Klinger et al., 1983; Leatherland, 1993; Pickering, 1993).

### 5.4 Evaluation of test diets

All test diets used in this study were more or less isocaloric in regard to total energy of dry weight and contained high protein levels (45-53%, Table 6). Despite this similarity, moist diets gave better results concerning growth, food conversion efficiency, disease susceptibility and

mortalities related to agonistic behaviour. Totally, three high quality dry pellets from different producers adequate for seabass-seabream culture were used during the rearing trials with juveniles and ongrowing common dentex. Therefore, this observation was not related to the formulation of a single producer, but seems to be generally associated with the feeding of commercially available dry pellets. This should be not much surprising, since the examination of foodstuffs shows, that the majority of feed formulations are very similar to each other (Jobling, 1983).

### **Influence of diet water content**

In contrast to energy content and proximate composition, the test diets differed considerably concerning their water content, energy density, particle size and texture. The impact of diet water content, energy density, and food particle size on feed intake, gastric evacuation time, digestibility of feeds, growth, and survival had been investigated in several studies before (Poston, 1974; Bromley, 1980; Garber, 1983; Jobling, 1981, 1986, 1987; Ottera et al., 1994). According to Jobling (1986, 1987), who found 5-10% lower digestibility coefficients in processed, formulated feeds than in natural food organisms, a better digestibility of moist diets was thought to be one reason for the better growth performance of juveniles fed with moist diets. Jobling assumed that high energy, small particles are emptied overrapidly from the stomach, leading to an overloading of the intestinal digestive capacity and a reduction in absorption efficiency. During experimental period 6, when the water content of commercial dry pellets was elevated from 8-10 (from producer) to 40%, comparable to the moisture of the moist diets used in this study, growth performance remained poor. This result is in agreement with the investigation from Garber (1983), who compared two moisture levels (4.7% vs 24.1%) in feed of juvenile yellow perch (*Perca flavescens*) and found similar gastric evacuation rates. Bromley (1980) who fed diets of similar nutrient content and different moisture ranging from 0-74% to juvenile turbot (*Scophthalmus maximus*) found no significant influence of dietary water content on growth, body composition, condition factor or food conversion: "It appeared that, as long as the basic nutrients were formulated so as to produce an adequate diet, the water content was immaterial". However, during weaning of cod (*Gadus morhua*) specific growth rates in the larger size group increased with increasing dietary water content, but this was not consistently found in the smaller size group and survival was not affected in both size classes (Bromeley & Sykes, 1985). Therefore, the authors conclude that dietary water content plays no major role in weaning success among juvenile cod.

### **Implications on water quality**

The feeding of moist diets is commonly accompanied by a relative high release of soluble compounds as well as particulate matter into the water. Such feed properties are especially important considering feed costs as major part of the total production costs and the increasing concern about environmental impact of aquaculture systems (Gowen & Bradbury, 1987; Alderman et al., 1994; Angel et al., 1995). Therefore, a preliminary estimation on the release of ammonia and suspended solids was conducted when different moist diets were fed to ongrowing dentex (EP 6).

The accumulation of total ammonia, nitrite and suspended solids are important characteristics for the management of aquaculture systems, in order to maintain favourable water quality

conditions. Food stability, its acceptance by the fish as well as the efficiency of food conversion do strongly influence the rate of accumulation under culture conditions for a given species. The total ammonia accumulation of 6 - 23 mg / kg / h during feeding determined during the present study, is well in the range of values for Sea bream of 90 g mean weight: 25.2 mg TAN/kg/h, after feeding (Porter et al., 1987) and Atlantic cod: 12.34-37.5 mg TAN /kg/h fed rations of 0.5-3.5 % bw (Ramnarine et al., 1987). However, in the present study, ammonia accumulation and not pure excretion was determined. Thus, ammonia accumulation includes also the release of ammonia from microbial degradation of food loss and faeces (nitrification).

The moist pellet prepared at site and the commercial pellet (Aqualim) prepared to contain a similar moisture (40%) expressed similar ammonia accumulation rates. Differences were difficult to detect, because of the high variability between, and the relative small number of samples. Since ammonia release by fish is known to follow a periodicity depending on various factors such as fish size, temperature and feed composition (Poxton & Allouse, 1987; Poxton & Lloyd, 1989; Porter, et al., 1987; EIFAC, 1986, Meyer, 1995), highest ammonia release may have occurred during a different period of the day.

The accumulation of suspended solids of 4.63 - 11.33 mg/l determined directly after feeding, when food particles were assumed to reach highest concentration in the fish tank, was considerably lower than the inorganic solid load of the filtered seawater supply (33 mg/l). This was rather unexpected, because the supply water appeared very transparent whereas the tank water after feeding contained many large food flocks easy visible by eye. The results revealed the difference between the small but dense inorganic particles in the sea and the relative voluminous food particles (from moist pellets) produced during feeding. Such quality differences between the different constituents of the suspended solid load will largely influence a potential impact on fish health. The efficiency of mechanical waste water treatment units is also largely influenced by the size class composition and quality of suspended solids (Cripps, 1991; Chen et al., 1993; Berg et al., 1993; Langer et al., 1996). Although the accumulation of suspended solids during feeding of moist pellets was relative low compared to the constant load of the supply water, the support of microbial growth on such particulate matter and the transfer of pathogens between fish of a culture unit have to be considered as potential hazards for the culture.

#### **Relationship between nutrition and disease susceptibility**

In the present study, dentex grown on dry pellets were more susceptible to disease. During experimental period 2 all groups fed with dry pellets got infected by a disease, whereas moist pellet fed fish remained healthy. Although there is general agreement that a well balanced diet is essential for adequate host defense mechanisms, few studies have dealt yet with the relationship between nutrition and disease resistance in fish (reviews are given by Landolt, 1989 and Blazer, 1992). The most important parameters affecting the immune response are vitamins, minerals and unsaturated fatty acids (Navarre & Halver, 1989; Scarpa & Gatlin, 1992; Obach & Laurencin, 1992; Olafsen & Hansen, 1992). The inclusion of fresh whole fish containing high levels of vitamins and HUFAs into the moist diet and the enrichment with a Vitamin mix and a HUFA oil may have strengthen the immune system of the common dentex under stressful conditions. Additionally it can be assumed, that the composition of commercial dry pellets available for bream and bass are not optimal composed for dentex.



Since this elevated disease susceptibility, when feeding exclusively dry pellets became clear already during the first rearing period and studies on vitamin, mineral and HUFA requirements are time intensive and will need several years, immunostimulation by  $\beta$ -glucans was thought a fast and efficient measure to reduce disease outbreaks in relation to stress under culture. The effects of two different immunostimulants on growth performance, mortalities and non-specific defense mechanism is discussed in the following chapter.

## 5.5 Immunostimulation by $\beta$ -1,3/1,6 glucans (EP 8)

### Effects on growth performance

The present study did not demonstrate any clear influence of the administered immunostimulants on growth performance and food conversion. As most other studies of immunostimulants in fish did not focus on growth yet, comparisons with the present study are limited. Levamisole, a synthetic compound with immunomodulatory capabilities (Jeney and Anderson, 1993b, Siwicki and Studnicka, 1994), has shown some growth enhancement in carp larvae (Siwicki and Korwin-Kossakowski, 1988), but no effects on survival and development were observed.

A considerable reduction in growth rate and food conversion efficiency, and the outbreak of infectious disease, occurred during weeks 5 and 6 of the experimental period. It is assumed that these were manifestations of the accumulated handling stress, caused by the weekly weighing procedure of this sensitive species. At least a two week interval between two handlings is recommended to allow full recovery from stress in salmonids (Pickering, 1993). Simulated handling stress in a growth model for large mouth bass (*Micropterus salmoides*) resulted in a 23 % reduction of growth over the season mainly due to reduced food consumption and increased respiration rates (Rice, 1990). Other rearing conditions which are known to influence growth performance (temperature fluctuations, reduced oxygen saturation, high un-ionized ammonia- and nitrite-content, extreme pH values) remained relatively constant and within favourable values throughout the entire experiment (Table 3). Increasing stocking density due to fish growth reached 7.0-14.4 kg/m<sup>3</sup>. This is regarded as an intensive, but not exceptionally high value for cultured sparids; thus it is unlikely that overcrowding has contributed to this sharp fall in growth performance. The partial recovery of growth rates during the last two weeks of the experiment, when the weekly weighing was stopped, support the supposed relationship between growth and handling stress.

### Effects on mortality rates

Simultaneous with the suppression of growth rates, five of the nine experimental tanks became infected by a protozoan parasite, which caused high mortalities. Because two replicate tanks of each immunostimulant group and one of the control tanks developed this infection, it is evident that the test groups fed the immunostimulant containing diets were not protected against the initial outbreak of the disease. However, once the fish were infected, the fate of the disease was related to the applied immunostimulants, with lowest cumulative mortality and the highest ratio of survivors in the MakroGard fish, followed by the VitaStim fish; control fish fared the worst (Figure 30, Table 16).

When comparing the cumulative mortalities as well as the percentage of infected fish of the different test groups, an elevated disease resistance was visible, which is in agreement with other investigations of  $\beta$ -glucans. One of the earliest reports of orally administered yeast glucans was by Raa et al. (1992), who tentatively reported highly reduced mortalities in juvenile Atlantic salmon after challenge with *Vibrio anguillarum* bacteria. High protection levels were found also by Siwicki et al. (1993), who tested 6 orally administered immunostimulants, which were fed during a 7 day period to ongrowing rainbow trout, prior to challenge with *Aeromonas salmonicida*. MacroGard achieved highest protection levels, expressed in a cumulative mortality of about 60% compared to the control mortality (100%). VitaStim was tested as a dietary supplement by Nikl et al. (1993) in different concentrations. They found that concentrations of 0.1% and 1% of food dry weight could significantly reduce mortalities induced by a furunculosis (*Aeromonas salmonicida*) challenge in chinook salmon. The concentration of VitaStim used in the present study (0.5% of feed wet weight) was within this range. A comparison of orally and intraperitoneally administered immunostimulants (Yano et al., 1989; Robertsen et al., 1990; Matzuyama et al., 1992) shows that both routes result in similar protection levels against different pathogens.

The outbreak of a protozoan disease in the present study was fortuitous because it allowed the first report of the effects of immunostimulants on a naturally occurring disease outbreak, and the first report of their protective effects against parasites. While most immunostimulation studies in fish apply challenge tests, exposing the pre-treated fish to a defined concentration of pathogens (by bath or injection), in the present study, fish were infected by variable concentrations of pathogens. Many protozoan parasites, if once established on a host fish, produce a high number of infective stages, which are distributed in the tank water. Thus, even one infected fish can increase the concentration of pathogens in a tank considerably. This led to the assumption that those tank populations, holding individuals with the relative weakest immuno competence got infected, regardless to the applied immunostimulant. The immuno-competence of the whole tank population was afterwards characterized by the percentage of infected and dying specimens (Table 16).

#### **Influence on blood parameter (non-specific immune response)**

At the end of the experiment, 5 blood assays were conducted to indicate differences in general health state (hematocrit, leucocyte numbers) and the activation level of the non-specific immune response (NBT-assay, lysozyme activity, total plasma protein) between fish administered the immunostimulant supplemented food and the control. The blood assays showed no clear activation of the non-specific immune response in immunostimulant treated fish.

Hematocrit levels are routinely recorded in fish health studies; elevated or decreased levels indicate a variety of diseases, stress effects or nutritional deficiencies (McLeay & Gordon, 1977; Obach et al, 1993). Hematocrit levels of about 40%, as measured in immunostimulant as well as control fish, were in the normal range for teleost fish (Filho et al., 1992). Leucocyte numbers are frequently monitored to estimate fish health state, influenced by disease, heavy metal exposure (Murad & Houston, 1988) as well as handling and social stress (Peters et al.,

1980, Peters & Schwarzer, 1985). In the present study, leucocyte numbers between the three replicate tanks of each treatment group differed considerably. Although the sample size per tank was rather small ( $n = 3-6$ ), this trend is at least suggestive. This trend can be explained by tank specific social stress, manifested by the occurrence of dominant, agonistic behaving individuals. The occurrence or absence of disease, including formol and copper - treatments, which had been shown to change several blood parameters in rainbow trout (Williams and Wooten, 1981) may also influence differences in leucocyte numbers between individual test tanks. Thus, possible effects of the immunostimulant treatments may have been covered.

Increased oxygen radical production of monocytes and neutrophils has been proven as a good indicator for an activation of the non-specific immune response in fish (Jeney & Anderson, 1993(a+b); Siwicki et al., 1993; Song & Hsieh, 1994; Jørgensen & Robertsen, 1995). In the present study, oxygen radical production was significantly reduced in the VitaStim fed fish and no effect in the MacroGard group was determined, indicating an inhibited or non-influenced, non-specific immune response in the two applied immunostimulants, respectively. Similarly, the lysozyme activity assay, another strong indicator for the activation of non-specific immune response (Engstad et al., 1992, Yoshida et al., 1995), did not differ between immunostimulated and control fish. Total plasma protein was the only blood parameter in the present study which indicated a trend in agreement with the observed protective effects of the immunostimulant treated fish during infection. A significant increase of plasma protein in the MacroGard treated fish and to a lower degree in the VitaStim treated group was found. This is in agreement with results from Siwicki et al. (1993), who found similar increased serum protein levels in MacroGard fed rainbow trout, parallel with elevated immunoglobulin levels.

Inconsistencies between different blood assays of the non-specific immune response have already been reported by Siwicki et al. (1993), who suggested that the normal biological variability is partly responsible for this effect. However, in the present study other effects seem to be more likely the cause for the observed non-activation of the immunostimulated fish. Adaptation processes in parts of the immune system after the prolonged application period of two months could explain the almost unaffected blood parameter. Regulatory mechanisms could reduce the non-specific immune response even below "normal" levels, when the activator (here the glucan) is no longer present. This could explain the significantly reduced NBT values in the VitaStim fed fish. Such effects were recently found by Yoshida et al. (1995), who fed African Catfish (*Clarias gariepinus*) over 45 days with MacroGard supplemented feeds. These authors found, that initially peaking values for glass-adherent, NBT- positive cells (day 30) decreased to baseline level at the end of the experiment (day 45). The effects of a prolonged application as well as the tank specific stress history can reasonably explain the inconsistent results of the blood assays measured at the end of the experiment, while obviously during an earlier period of the experiment (disease during week 5 and 6) an activation of the non specific immune response was achieved by the administered immunostimulants.

### Conclusions and outlook

This experiment on immunostimulation in juveniles demonstrates the strong interrelationship between cumulative stress, suppressed growth performance and increased disease susceptibility. The initial question if fast growing juvenile common dentex could be somehow

protected from sublethal infections by the uptake of immunostimulating  $\beta$ -glucans and therefore expressing a better growth performance could not be answered positively here. Results support the possibility of using  $\beta$ -glucan preparations as a feed supplement to prevent fish stocks from losses by protozoan infections.

Further work is needed to understand the modification of the non-specific immune response in fish populations under aquaculture conditions where fish are normally exposed to a variety of stresses and pathogens. Duration of application, possible adaptation processes of the cell-mediated immune response as well as interaction with stress responses under commercial culture conditions have to be defined to allow a reliable application of  $\beta$ -glucans in aquaculture.

## 5.6 Morphometric growth pattern and organo-somatic indices

### Meristic characters

During the ontogeny of fishes, strong changes in external appearance and relative dimensions of body parts do occur. Such allometric growth patterns reflect changing life styles and requirements, which are clearly expressed during the larval life stage. However, changes continue to occur to a lesser extent also in juveniles and adults (Balbontin et al., 1973; Fuiman, 1983; Baldwin et al., 1991; Fukuhara, 1992; Otterå & Folkvord, 1993; Osse & Boogaart, 1995). The growth pattern and changes in morphometric characteristics established in this study provide a data base for the description of the development in common dentex under culture conditions. The length - weight relationship described by a power function ( $TW \text{ (mg)} = 0.00944 * TL \text{ (mm)}^{3.1045}$ ) allows to back calculate weight from length data in fish ranging between 0.0026 g and 4000 g, including the largest life cycle stages in culture. The strong linearity found for the relationship between all measured length characters and total length allows, a) to back calculate these variables from values of total length and b) to compare growth patterns of this study with results from other studies (e.g. various culture conditions or field situations).

In about 20 fishes (20-40 mm TL), the deviation of the linear relationship between eye diameter and total length was observed (Figure 39). This could be explained by a systematic measurement fault. This suggestion is supported by the fact that all these fish were measured at one time, and the line fitted through these points is parallel to the regression line determined for the majority of the fish. However, a biological cause can not be excluded to be the reason for such a difference in relative eye diameter between fishes reared under similar conditions

The analysis of allometric growth revealed markedly changes of body shape during development of juvenile common dentex. In fishes up to 100 mm TL, the relative head length and eye diameter were rapidly decreasing with growth. Until a size of about 50 mm TL a faster growth of the posterior part of the body, characterized by the pre-anal length, became obvious. Such a positive allometry of the head (including the eye) is typical in early life stages, when feeding and respiratory functions are a priority and the full development of inner organs such as the intestine (Osse & Boogaart, 1995) must be adjust first to changing life style. In larger fish the growth rate of the head slows down compared to other parts. Especially the tail region

seemed to grow faster in fishes above 100 mm TL. The faster growth of the posterior part of the body, namely the tail, is characterized by the relative increase of the post-anal length during this developmental phase. During the same period, a change of the tail fin shape, indicated by the decrease of the relative fork length can be assumed. The accelerated growth of the locomotory apparatus in fish of about 100 mm TL (about 3-4 months after hatching) indicates a change in habitat and/or target prey organisms of this species at the end of the first summer of their life (August - October).

The relationship between the size of mouth opening and the type and preferred size of food organisms or particles has been reviewed by Hyatt (1979). The predator : prey ratio depends also on mouth gape size and body depth in cannibalistic species (Otterå & Folkvord, 1993; Bry et al., 1992; Parazo et al., 1991). The maximum mouth opening (vertical distance between the tips of upper and lower jaw) determined during this study decreased from about 15-18% TL in juveniles (< 50-100 mm TL) to 10-13% of TL in ongrowing fishes (> 100 mm). This corresponds well with the generally decreasing amounts of food intake observed in larger fishes (Zitat). Thus, smaller fishes can ingest relative larger food particles, fulfilling their demand in nutrients and energy with less frequent prey catches. In larger fish, hydrodynamics becoming more important and the head and mouth are relative smaller, resulting in a more streamline body-shape (Osse & Boogart, 1995). It becomes clear that allometric growth supports an optimal energy balance during different life stages considering scaling effects (Giguere et al., 1988), predator- prey relationships including interspecific predation (Otterå & Folkvord, 1993), hydrodynamics of fish movement (Osse & Boogart, 1995) and variable environmental parameters such as water temperature and currents (Houde, 1989). The biological variability of growth pattern in fishes is a known phenomenon and probably an adequate tool for the adaptation to rapidly changing environmental conditions. Morphological differences commonly found in hatchery reared and wild fish can most likely be explained by such adaptation effects to a different environment (Baltontin, 1973).

Most studies on allometric growth pattern focused on larval and early juvenile stages. The change of body shape in larger fishes is more moderate, but results obtained during this study reveal that valuable information on changing life requirements can be extracted from their analysis. However, the relative small sample sizes in the larger fishes as well as the general high variability of the examined characters, do not allow to determine distinct developmental stages from the data set of this study.

### Organo-somatic indices

Similar to morphometric development, the organo-somatic indices describe the relative growth of several organs in relation to relative weight of fish and it is believed that these ratios reflect also the response to culture conditions. The amount of fats stored, in particular the mesenteric fat, can be considered as an indicator of the balance between food nutrient supply and total digestible energy. MFI values during this study ranged from almost 0 to 2.4 %, well in the range of juvenile red-spotted grouper (*Epinephelus akaara*) grown in net cages under variable feeding frequencies (Kayano et al., 1993). In the later study, a feeding frequency of 6 times a day, similar to 5 times a day used in the present study, resulted in highest muscle mass and highest levels of muscle protein, whereas muscle lipid decreased with increasing feeding

frequency and the intraperitoneal fat deposition tended to increase, reaching highest values of  $1.37 \pm 0.64$  %. This observation supports the results reported by Tibaldi et al. (1996) who found relative low body lipids (3.9-6.2%) in juvenile common dentex grown under favourable conditions compared to other marine species currently being farmed in the Mediterranean area such as seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). These authors suggest that the low body lipid content of the common dentex may represent an important quality trait, because there is an increasingly demand for low lipid products.

The Liver Somatic Index (LSI) is a more sensitive indicator in response to food intake and food quality characteristics than the determination of the general condition factor. However, liver condition not only reflect short-term energy intake but also reproductive and temperature-related metabolic demands (Adams & McLean, 1985). The LSI determined during this study (LSI = 0.8 - 2.8 %) was in the range of those found in other carnivorous fish, such as rainbow trout (*Oncorhynchus mykiss*: LSI = 1.05-2.06 %) (Haider, 1984) and grouper (*Epinephelus akaara*: LSI = 2.13-2.94%) (Kayano et al., 1993). The change of the LSI with growth, shown by Haider (1984) for carp and rainbow trout, was not clearly expressed as documented for the common dentex during the present study.

The length of the intestine is commonly related to the type of food consumed under natural conditions. Relative intestine length (intestine length / standard length) in herbivorous fish such as *Ctenopharyngodon idella* (2.5) and *Hypophthalmichthys molitrix* (4.6-7.1) are considerable higher than values from omnivorous species while the shortest intestines are found in strictly carnivorous species : *Salmo salar* (0.73-0.8), *Micropterus salmoides* (0.7-0.9) (Weatherley & Gill, 1987; Kapoor et al., 1975). Ribble and Smith (1983) described the allometric relationships between intestine length (y) and total fish length (x) for 11 species of (mostly carnivorous) fish from seven families by means of a single equation;  $Y = 0.08 * X^{1.42}$ . Thus, intestine length increases at a relatively higher rate than fish length. Even among carnivorous species intestine length is often related to size of prey, being greater in those that consume smaller animals. The relative short intestine of juvenile and adult common dentex (about 0.4-0.9 % of total length) is typical for strictly carnivorous species. Changes in relative intestine length were not observed in specimens between 100 and 500 mm TL, indicating relative constant nutritional requirements during this developmental stages. However, the inner surface area or the enzymatic composition of the digestive system may be indicative of changing nutritional requirements, despite the isometrically length growth of the intestine.

Relative gonad weight was determined in a few specimens only. In juveniles between 200 and 400 mm, gonad weight accounted for less than 0.5% of the body weight and increased fast in larger specimens reaching values up to 2 to 4.5 %. Due to the small sample size no reliable information on time and degree of maturation can be gained from these results.

## 5.7 Abnormal developments

Skeletal deformations are commonly related to non-optimal environmental conditions, often during sensitive developmental periods (Daoulas et al., 1991; Akiyama et al., 1986, Lindsey & Arnason, 1981; Pohl, 1990), due to nutritional deficiencies (review by Tacon, 1992) and

sometimes also induced by practices of population genetics such as excessive inbreed (McAndrew et al., 1993). Since all individuals of any species carry some abnormal alleles, which are usually hidden, the probability that such alleles are present in their homozygous state is increased if mating between closely related individuals occurs. This is commonly the case when the broodstock of a hatchery is renewed only from hatchery reared specimens. In this study all reproductive cycles originated from gametes which were obtained from first generation parental fish. Thus, the role of genetic effects on the prevalence of abnormal developments should not be elevated compared to wild fish. An increased rate of abnormal developments should therefore be related to unfavorable environmental conditions such as temperature, mechanical stress and illumination or the applied nutritional regime. However several pathogens have also been reported to cause morphological anomalies in fish (Akijama et al., 1986, Lindsey & Amason, 1981; Lom et al., 1991; Pommeranz, 1974) and even in wild fish populations a relative high percentage of specimens with skeletal anomalies have been found (Möller, 1983, Pohl, 1990).

The lordosis of the vertebral column is frequently related to an uninflated swim bladder in hatchery reared marine fishes (Kitajima et al., 1981; Boglione et al., 1995; Weppe & Bonami, 1983). Lordosis observed in juvenile dentex during this study also occurred in specimens without inflated swim bladder. However, the lordotic deformations in juvenile dentex of the first production series was expressed only in slight bents of the vertebral column near the head, which was most likely independently caused from the lack of a timely swim bladder inflation. Typically a strong curvature of the middle part of the vertebral column causes the fish to swim in an abnormal position. Additionally these fish expressed a variety of deformations, such as the twisted operculum syndrome or fused vertebrae, which indicates that also other causes did manipulate the development during this rearing period. Similar opercular deformations were reported from Barahona-Fernandes (1982) in laboratory reared European seabass (*Dicentrarchus labrax*). These authors found that such deformations did not reduce growth, but there was evidence that it lowers resistance to oxygen depletion and increase susceptibility to myxobacterial infections (Paperna et al., 1980 cited in Barahona-Fernandes, 1982). These results correspond to some extent with the results of this study, where growth rates were still high in deformed fish (EP 1+2). Although fish with a high prevalence of skeletal deformities became infected, such outbreaks have also been observed in normally developing specimens and these were close related to nutritional and handling stress. The skeletal deformations observed during the rearing periods were most likely related to inadequate larval rearing conditions. The requirements of this species for its larval rearing are still very poorly known as culture trials with dentex have just recently started. Early life stages of marine fish species are generally highly sensitive against deviations from optimal rearing conditions, as well documented from established aquaculture systems.

The enlargement of scales, observed during one production series is not commonly reported in other fish species. However, the enlargement of scales in mirror carp is a well known phenomenon, which is based on the selective breeding of specimens with enlarged scales (Zitat). Assuming similar mechanisms in the common dentex, the enlargement of scales is genetically rather than environmentally caused.

## 5.8 Disease syndromes related to culture conditions

### Swimbladder stress syndrome (SBSS)

The positive buoyancy observed in stressed juveniles and especially in broodstock fishes reminded a phenomenon, described in the literature as the swim bladder stress syndrome (SBSS). In larval fishes the hyperinflation of the swimbladder caused high mortalities in hatchery reared sea bass larvae (*Lates calcarifer*, *Dicentrarchus labrax*) (Bagarinao & Kungvankl, 1986; Johnson & Katavic, 1984). The authors suggest that SBSS could be caused by numerous stress inducing factors such as handling, crowding, oxygen depletion, unfavourable water quality parameter and nutrition. Stressed larvae showed disproportionately large swimbladder volumes relative to larval size. Larvae with 4 x the normal gas volume could still swim, while those with 10 x more gas in the swimbladder as healthy larvae were floating at the water surface (Bagarinao & Kungvankl, 1986).

It is assumed that stress hormones (Catecholamines) do influence gas deposition in the swimbladder. Kolbeinshavn & Wallace (1985) described the SBSS in Arctic charr (*Salvelinus alpinus*) juveniles, which is reported to be induced by inadequate water depth. These authors divide the symptoms of this syndrome into 4 progressive stages: (2) fish are swimming at the surface with their backs exposed, (3) fish are swimming intermittently and erratically on their sides. These stages were also observed over an extended time period in dentex broodstock fish, which had been newly transferred from a net cage system into a rearing tank of 90 cm water depth. One large genitor died after about 2 months showing such behaviour and no external or internal signs of disease or abnormal organ development. Although measurements of swimbladder volumes were not possible during this study, it is assumed that ongrowing and adult common dentex are susceptible to the SBSS syndrome.

### Gas bubble disease

Gas bubble disease (GBD) occurred when ongrowing dentex from hatchery production were accidentally stocked in supersaturated water of 110% total gas pressure (TGP). Weitkamp & Katz (1980) reviewed the literature on the effects of dissolved gas supersaturation. The authors describe that bubbles appear first in the unpaired fins and the eyes, causing exophthalmia in salmonids, which confirms well with signs of GBD observed during the present study. Also the fast death of Atlantic menhaden after 24 hours exposure to 110 % gas saturated water, as well as the colour change reported from these fish was very similar to the development of this disease in common dentex (McLeod, 1978). The susceptibility to gas supersaturation varies between species and fish life stage. Gray et al. (1985) found that the 96 h LC<sub>50</sub> was 124.8 % for fingerling striped mullet (*Mugil cephalus*) and only 116% for seabass (*Dicentrarchus labrax*), while post larvae of the two species expressed a similar susceptibility.

Acute mortality is caused by gas bubbles, which block the blood flow in important blood vessels such as the gill arterioles or the bulbous arteriosus (Weitkamp & Katz, 1980). Tucker (1993) described that chronic gas bubble disease is associated with hyperinflation of the swim bladder and extravascular emboli in the gut and buccal cavity. Similar signs have been reported from larval striped bass (*Morone saxatilis*), causing mortalities at total gas pressures as low as



105-106% (Cornacchia & Colt). The hyperinflation of the swimbladder is also a typical sign of the Swim Bladder Stress Symptom described above. A relationship between these both syndromes maybe considered.

There is evidence that the common dentex is particularly susceptible to gas supersaturated water. Black seabream (*Spondyliosoma cantharus*), which were kept in similar gas supersaturated water did not express any acute signs of GBD, even after a prolonged period. Additionally, from an Italian hatchery it was reported, that mainly common dentex died during a period of gas supersaturation, whereas other sparids such as the gilthead seabream (*Sparus aurata*) or the red seabream (*Pagrus major*) were not affected (Italo Di Maria, pers. com.).

## 5.9 Conclusions and outlook

High growth potential and highly efficient food conversion is always appreciated by aquaculturists in order to optimize operational costs for sustainable development. It is therefore of interest to note that the growth and food conversion determined in juvenile (0+) dentex exceeded comparable values of already well established aquaculture species cultured in the Mediterranean area. The rapid growth to marketable size (300-500 g) of *Dentex dentex* makes this species an excellent aquaculture candidate for future development of the industry.

High mortality rates during the first year of growth in captivity were mainly caused by agonistic biting behaviour between tank - mates. During the present study it was shown that agonistic behaviour could be largely controlled by modifications of culture management and rearing technology. Furthermore, adequate dietary regimes (resulting in best growth performance) considerably reduced aggression compared to unfavorable diets. It should be stated that behavioural aspects are of utmost importance when considering to develop a culture technology for a given species.

Different commercial dry pellets, composed to fulfill the requirements of related species (*Sparus aurata*) were fed to common dentex juveniles and ongrowing fish. Although commercial dry diets were accepted from juvenile life stages on, many parameters of culture performance were negatively affected compared to groups fed with moist diets, the latter being prepared close to the natural diet of this species. Growth rates and food conversion efficiency were markedly reduced when commercial dry pellets were fed exclusively, and during one rearing trial disease susceptibility related to such a dietary regime was increased. Therefore, it is suggested that commercial dry diets available for related species are nutritionally imbalanced with regard to the dietary requirements of common dentex.

The frequent outbreak of infectious diseases during the rearing trials of this study were mainly induced by intense handling stress due to experimental weighing schedules. Protozoan parasites, located on gill and skin surfaces were assumed to be the causative agents of these diseases. The application of standard treatments (antibiotics, antiparasitics) was successful for controlling the infections.

Immunostimulation by orally administered  $\beta$ -1,3/1,6 glucans has been demonstrated to be a possible measure to reduce mortalities caused by infectious diseases in dentex juveniles. However, disease outbreak was not reduced in groups treated with the immunostimulant, compared to the control group, but subsequent mortalities among infected fish groups were markedly lower in immunostimulated populations. Therefore two aspects must be considered: (a) immunostimulation can be an efficient health management tool for the culture of dentex, particularly during periods of intense handling stress, (b) immunostimulant application will only assist in minimizing losses due to prevailing infections if stress is minimized and culture techniques are adjusted to fully meet the requirements of this species.

The occurrence of skeletal deformation during this study was mainly related to unfavourable larval rearing condition (intensive rearing). Fry produced by the mesocosmos rearing technique, which relies on natural zooplankton as feed source at initial feeding, expressed abnormalities at low prevalence. The kind of abnormalities found during this study were not restricted to common dentex, but were reported to occur also in variety of other fish species.

## Outlook

Besides the favourable growth performance experienced during this study and the high wholesale prices obtained for the common dentex throughout the Mediterranean region, the development of large scale culture of the species will largely depend on several practical and scientific criteria:

(a) improving handling procedures to minimize stress to this very sensitive and nervous species

(b) controlling mass reproduction by developing photo- and temperature - related rearing schedules, making year round production possible

(c) developing adequate feeds that do not only fulfill the nutritional requirements of the species, but affects satiation so as to reduce the agonistic and "cannibalistic" behaviour of this species.

All three aspects will become more important if culture technology for this species go through the scale-up phase, where adequate mass handling becomes a crucial operational criteria for success.

Effects, such as reduced growth rates, low food conversion efficiency and increased disease susceptibility, which were related to the feeding of commercial dry pellets in the present study, have been caused by inadequate amounts of dietary essential fatty acids in several species (Koven et al., 1990 - *Sparus aurata*; Tacon, A.G.J., 1992 - review; Kanazawa, 1985 - *Pagrus major*; Watanabe, T., 1982 - review; Bell et al., 1985 - *Scophthalmus maximus*). The moist pellets used in the present study contained considerable amounts of marine fish (known to contain high amounts of Highly Unsaturated Fatty Acids, HUFAs), and was additionally enriched with a concentrated HUFA oil mixture (1.5% of food wet weight). In contrast, commercial dry pellets for marine fishes are supplemented with 1.5 - 2% HUFA for fingerling and only 1% in feeds for ongrowing fish. Such HUFA levels have been recently shown to be insufficient for red seabream (*Pagrus major*), when feeding diets of high total lipid content. Under such conditions HUFA - requirements were elevated to 3.7% (Takeuchi et al., 1992).

Since the test diets used in the present study contained relative high total lipid level (12.6 - 20%), the respective HUFA content was possibly insufficient to fulfill the requirements of the common dentex. However, since HUFA requirements differ considerably between fish species (Watanabe, 1982), a thorough study is needed to prove the influence of dietary HUFA content related to dietary lipids on growth and health performance of common dentex.

The adjustment of culture technology and management with regard to species specific behavioural pattern, such as agonistic behaviour and biological traits such as the sensitivity to handling stress and gas supersaturation will help to further improve culture performance of this species. Tank system design largely influences the behavioural performance of a species, therefore different types of circular tanks systems, raceway tanks and net cages have to be investigated for their suitability in mass culture of common dentex. Especially floating net cages, which are commonly employed for grow-out stages of marine species in the Mediterranean region may cause difficulties due to problems in controlling agonistic behaviour. This difficulty is particularly stressed considering that size grading, one of the most efficient means for controlling aggression in net cage culture, is a labour-intensive and therefore costly procedure, which additionally causes considerable handling stress to fish. The relationship between stress, induced by routine handling, and disease susceptibility, as well as the identification of pathogens important in this species require considerable research effort to allow the reliable mass rearing of common dentex. Temperature and water quality preferences of this species have to be defined for adequate site selection programs, and the establishment of seasonally dependent rearing schedules, which largely influence production efficiency. The identification of optimal stocking densities for separate size classes will considerably help to improve production efficiency.

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